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Photosynthesis of Lamina and Sheath of Barley Leaves

BY

GILLIAN N. THORNE

(*Rothamsted Experimental Station, Harpenden, Herts.*)

With one Figure in the Text

ABSTRACT

Apparent photosynthesis, in mg. CO_2 absorbed per dm.^{2} per hour, of the sheath and enclosed stem of a barley leaf was about 50 per cent. of that of the lamina of the same leaf, when the photosynthesizing area was measured as one side of the lamina and the outer exposed surface of the sheath. Apparent photosynthesis of a particular lamina or sheath was about 70 per cent. of that of the one above on the same stem.

Respiration per dm.^{2}, though not per g. dry weight, of sheath with enclosed stem was greater than of lamina in one experiment done with low-intensity illumination so that true rates of photosynthesis of lamina and sheath were similar. Differences in respiration rates per unit area of laminae and sheaths probably accounted for most of the greater apparent photosynthesis of the former in other experiments done with higher intensity illumination.

It is suggested that for growth-analysis studies the size of the photosynthetic system of cereals should be measured as that of one side of the leaf laminae plus the outer surface of the combined leaf sheaths. In the later stages of growth the surface area of exposed stem and peduncle should also be included.

INTRODUCTION

THE rate of growth of a plant in dry weight depends on its photosynthetic capacity, and in growth analysis this is usually measured by the total leaf area (Watson, 1952). Leaf area of cereals has customarily been taken as that of the leaf laminae, ignoring the sheaths and stems, but this is not correct because the sheaths also photosynthesize. Thus the grain yields of wheat and barley plants with ears shaded and all the leaf laminae removed or shaded from the time of ear emergence was between 22 and 55 per cent. of that of unshaded intact plants (Boonstra, 1929; Archbold, 1942; Asana and Mani, 1950). This yield of grain is an approximate measure of assimilation in the leaf sheaths and stem because the products of current assimilation after ear emergence migrate to the developing ear (Archbold and Mukerjee, 1942).

If leaf-lamina area is measured as one side of the lamina and the area of the sheath is taken to include any exposed green surface of stem or peduncle, which is small compared with true sheath except at the end of the growth period, the area of sheath varies from a negligible fraction of the total green area soon after germination to almost 100 per cent. just before maturity (see figure). The proportion at any time is similar for different cereals and was unaffected by nitrogenous fertilizer in two experiments and only slightly

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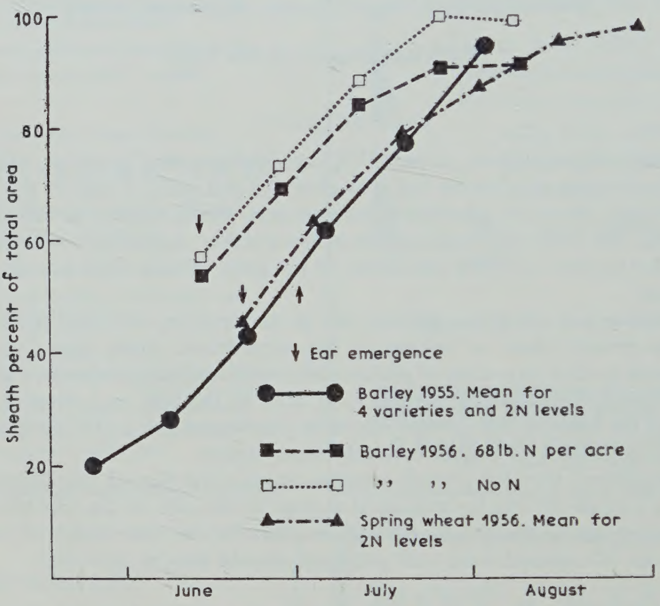
affected in another, where nitrogenous fertilizer more than doubled the yield. Sheath accounts for about half the total assimilating area at the time of ear emergence, and so is an important fraction of the total area during grain development. For comparative purposes, ignoring the sheath before ear emergence will not be so very serious because at this time it is a smaller proportion of the total area and this proportion is apparently not much affected by external conditions. But when comparisons of photosynthetic efficiencies are made between cereals and dicotyledons the sheath should always be included in the estimate of leaf area of the cereals.



Contribution of sheath, exposed stem and peduncle to the total leaf area of cereals in the field.

To obtain more detailed information about the relative contributions of photosynthesis of sheaths and laminae to that of the whole plant, the uptake of CO_2 by single laminae or portions of sheaths and enclosed stem was measured with an infra-red CO_2 analyser.

METHODS

Measurements of photosynthesis were made on laminae and sheaths of main shoots of barley plants (variety Plumage-Archer) near the time of ear emergence. The plants were grown in pots of soil and, while the measurements were being made, were illuminated with natural daylight in an unheated greenhouse in Expt. 1–3; in Expt. 4 the temperature in the greenhouse was maintained above 15° C. In Expt. 1 the natural daylength was extended with artificial light during part of the growth period to ensure stem elongation. Photosynthesis of the lamina and sheath of the four youngest leaves was measured. Four comparisons were possible on one day and replication was obtained from runs done on successive days, using uniform batches of plants. The leaves used, the number of replicates, the time and dates, and the mean temperatures of the four experiments are shown in Table 1. The temperatures were obtained by averaging readings from thermograph charts.

TABLE 1

Experimental Details

Experiment	Number of replicates	Time G.M.T.	Date	Mean temperature, °C.	Number of green leaves per shoot*	Leaves measured*
1	4	09.00–14.00	25–26 May	15	6	2
2	4	09.00–14.00	10–14 June	28	6	1 and 2
3	7	10.00–15.00	9–15 July	27	5	2 and 3
4 assimilation	5	12.00–15.00	8–12 October	25	6	3 and 4
4 respiration	5	18.00–06.00	8–12 October	18	6	3 and 4

* Leaves numbered from flag leaf (= 1) down stem.

Photosynthesis was measured from the difference in CO_2 concentration of air before and after passage over the leaf. The concentration was recorded for each leaf during 10 minutes in every hour. The assimilation chambers, constructed from glass tubing of 1·6 cm. internal diameter, were 36 cm. long for the laminae and 8 cm. long for the sheaths (13 cm. for the flag-leaf sheaths), and the rate of flow through each was 200 ml. per min. The lamina was held in the base of the assimilation chamber between two pieces of a split rubber bung and air was sucked over it and out to the analyser via a narrow tube inserted in one piece of the bung. The stem was held at each end of the assimilation chamber by similar split rubber bungs, and air entered at the top and left at the base via narrow tubes. Leaves were enclosed in the assimilation chambers for about 7 hours in Expt. 1–3 and 24 hours in Expt. 4. The mean rate of photosynthesis for the same 5-hour period in the middle of the day was calculated for each tissue and each run in Expt. 1–3. In Expt. 4 mean photosynthesis rate was calculated from only 3 hourly values and mean respiration rate from 12 hourly values (Table 1).

At the end of the experimental period the portion of the lamina or sheath that had been enclosed in the assimilation chamber was removed, measured, and dried at 100° C. to give dry weight per unit area. The area of one side of the lamina was measured with a planimeter from a contact print made on light-sensitive paper and sheath area was taken as the product of length and mean circumference, length and diameter being measured with a millimetre scale. Photosynthesis and respiration rates were expressed as mg. CO_2 absorbed or produced per $dm.^2$ of lamina or sheath surface per hour. Respiration was also expressed per gram dry weight, the sheath dry weight including that of the enclosed stem. Rates of respiration in the light were estimated by correcting respiration measured at night by the difference between the mean temperatures of the periods during which respiration and photosynthesis were measured, assuming $Q_{10} = 2$. These were then added to apparent photosynthesis to give estimates of true photosynthesis.

RESULTS AND DISCUSSION

The apparent rate of photosynthesis of the sheath was always less than that of the corresponding lamina (Table 2); it varied from 44 to 55 per cent. of that of the lamina except for leaf 4 in Expt. 4 when photosynthesis of the sheath was negligible. It was consistently low in all runs of this experiment; in two there was no CO_2 absorption and in one there was slight CO_2 production in the light. This sheath, though still green, must already have been senescent.

TABLE 2

Rate of Apparent Photosynthesis of Lamina and Sheath of Barley Leaves, mg. CO_2 per $dm.^2$ per hour

Leaves numbered from flag leaf (= 1) down stem

	Expt. 1	Expt. 2		Expt. 3		Expt. 4	
	Leaf 2	Leaf 1	Leaf 2	Leaf 2	Leaf 3	Leaf 3	Leaf 4
Lamina . .	7·0	8·8	5·6	8·3	6·0	4·4	4·6
Sheath . .	3·3	3·5	2·4	3·7	2·6	2·5	0·4
S.E.. . .	0·25	0·62		0·64		0·54	

Apparent photosynthesis appeared to be greatest in the most recently produced leaves (Table 2). The rate of apparent photosynthesis of a particular lamina or sheath was about 70 per cent. of that of the leaf above. In Expt. 4, in which the leaves used were older than in the previous experiments, the rate of photosynthesis of the laminae of leaves 3 and 4 were similar but photosynthesis of the sheath of leaf 4 was only 15 per cent. of that of leaf 3. More work is needed to investigate the relative rates of photosynthesis of all the leaves on a shoot at one time, to determine how photosynthesis of a single leaf varies with age, and whether it falls to zero while barley leaves are still green. Photosynthesis at any one time of the successive leaves on shoots of other species decreased from the first to the last formed leaf (Singh and Lal, 1935), though it may also be low in very young leaves (Richardson, 1957). The effect of position was smaller in potato than in barley; rates of photosynthesis of the 4th and 12th leaves below the terminal cluster of leaves were 92 and 66 per cent. respectively of that of the 3rd leaf (Chapman, 1951).

Variability between replicate runs was high in the present experiments, especially in those on older leaves. Some of this variability was caused by changes in external factors, of which light intensity was probably the most important, and this could be decreased by measuring photosynthesis in a controlled environment under artificial light, but in these conditions light intensity might be limiting photosynthesis. For measurements in natural conditions large numbers of replicates are always essential because of the large variations in photosynthesis which apparently are not entirely attributable to changing external factors (Chapman and Loomis, 1953).

The rates of true photosynthesis of the lamina and sheath of leaf 3 in Expt. 4 were approximately equal (Table 3). In leaf 4, in which apparent photosynthesis rate of the sheath was only 9 per cent. of that of the lamina, true photosynthesis rate of the lamina was almost twice that of the sheath. In both leaves the high rate of respiration per unit area of the sheath compared with the lamina was due to the greater dry matter per unit area of the former, which resulted from the inclusion of the enclosed stem with sheath. Respiration rates of lamina and sheath on a dry-matter basis were similar (Table 3).

TABLE 3

Expt. 4. Rates of Respiration (at 18° C.) and True Photosynthesis of Lamina and Sheath of Barley Leaves, mg. CO$_2$ per hour

Leaves numbered from flag leaf (= 1) down stem

	Respiration				True photosynthesis	
	per dm.^{2}		per g.		per dm.^{2}	
	Leaf 3	Leaf 4	Leaf 3	Leaf 4	Leaf 3	Leaf 4
Lamina . . .	0·6	0·9	2·6	2·9	5·5	6·1
Sheath . . .	2·3	1·8	3·2	2·3	6·5	3·5
S.E. . . .	0·08		0·23		0·71	

The ratios of rates of apparent photosynthesis of sheath to that of lamina in Expts. 1–3 were similar to that of leaf 3 in Expt. 4 but the absolute rates were greater, presumably because of the higher light intensity in June and July than in October. True photosynthesis could not be calculated as in Expt. 4 because respiration was not measured, but approximate estimates were made by assuming that respiration rates per unit dry matter of lamina and sheath were equal and the same as in Expt. 4, 2·8 mg. CO$_2$ per g. per hour at 18° C. From this figure and the dry weight per unit area of the leaves used in Expts. 1–3, respiration per dm.^{2} at the temperature of the photosynthetic measurements, and hence the rate of true photosynthesis, were calculated. The rate of true photosynthesis of the sheath, estimated in this way, was about 85 per cent. of that of the lamina (Table 4). The actual rate of respiration may have been slightly greater than the calculated value (Richards, 1932; Pope, 1933); if so, provided that the rate remained the same for lamina and sheath, true photosynthesis rate of the sheath would have been more nearly equal to

TABLE 4

Estimated Rates of True Photosynthesis of Lamina and Sheath of Barley Leaves, mg. CO$_2$ per dm.^{2} per hour

Leaves numbered from flag leaf (= 1) down stem

	Expt. 1	Expt. 2		Expt. 3	
	Leaf 2	Leaf 1	Leaf 2	Leaf 2	Leaf 3
Lamina	7·9	11·0	8·1	10·3	8·3
Sheath	5·8	8·6	7·8	7·9	7·9

that of the lamina. Thus the approximate estimates made for Expts. 1–3 support the experimental evidence from Expt. 4 that the rate of true photosynthesis per unit area of the sheath of a barley leaf is of the same order as that of the lamina.

As the leaf sheaths of cereals have a true rate of photosynthesis per unit area similar in magnitude to that of the leaf laminae, the area of the sheaths should be included with that of the laminae in estimates of total leaf area for the purpose of growth analysis, and particularly as a basis for calculating net assimilation rate. If this is not done the values obtained will greatly overestimate the efficiency of cereal leaves in dry matter production. Previous measurements (Watson, 1947) have shown that net assimilation rate of cereals based on lamina area is much less than that of dicotyledonous crops growing in the same conditions. This comparative inefficiency of cereal leaves becomes even more pronounced when both laminae and sheaths are included in the measurement of total leaf area.

ACKNOWLEDGEMENTS

Measurements with the infra-red CO_2 analyser were made with the help of Dr. P. C. Owen whom the author wishes to thank.

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Investigations on Growth and Metabolism of Plant Cells

VII. Sources of Nitrogen for Tissue Cultures under Optimal Conditions for Their Growth

BY

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With one Figure in the Text

ABSTRACT

Tissue cultures from explants of carrot root and potato tuber, stimulated into rapid growth by the addition of coconut milk or *Aesculus* liquid endosperm to the medium, become to a certain degree heterotrophic for nitrogen. Maximum growth-rates are attained only when nitrate is supplemented with various reduced nitrogen compounds. The effects of casein hydrolysate, amino-acid mixtures, ammonia, tryptophan, urea, and allantoin have been investigated, and their possible biochemical roles are discussed.

NATURE OF THE GROWTH-PROMOTING SUBSTANCES IN COCONUT MILK

WHEN coconut milk or a number of other liquid endosperms and plant extracts are added to an otherwise complete nutrient medium for the aseptic cultivation of explanted tissue, they induce rapid cell proliferation in the tissue of certain plants (Caplin and Steward, 1948; Steward and Caplin, 1951; Steward and Shantz, 1955; Duhamet, 1951). Tissue explanted from the secondary phloem of carrot root has been used extensively to study this effect (Caplin and Steward, 1949; 1952).

Fractionation of these endosperm preparations in an effort to isolate the growth-promoting principles, has provided a number of concentrated fractions, as well as purified compounds, which exert their stimulatory activity only when the nutrient medium is supplemented by a source of reduced nitrogen, such as casein hydrolysate. The purpose of the present paper is to present further observations on this aspect of the growth of tissue cultures to supplement the more casual references to this which have already been published (Shantz and Steward, 1952; Steward and Shantz, 1954).

Early work on the fractionation of coconut milk soon showed that certain fractions were of high potency in the carrot-tissue growth test, but they only expressed this activity after recombination with other fractions which were known to be rich in free amino-acids. This latter type of fraction could be effectively replaced by casein hydrolysate, which furnished a more standardized source of nitrogenous supplement. Since that time nearly all experiments on the growth of tissue cultures have been replicated in two different basal media, one of which contains a supplement of enzymatic casein hydrolysate. Several hundreds of experiments during this period have furnished consider-

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able data on the effects of casein hydrolysate and other nitrogenous nutrient supplements on the aseptic growth of plant tissue cultures under various conditions of treatment.

One factor which complicates the study of the growth response is the inherent variability of explants from different carrot roots, even though these roots may be drawn from the same variety, grown under the same conditions in the same locality. It has been shown, for instance (Steward and Shantz, 1954), that when grown in a medium which contains coconut milk, 100 cultures from explants of the same root, allowed to grow to 40 times their original size, had a standard deviation in average final fresh weight of only ±5 per cent., while 1,000 explants from 200 different roots, grown under identical conditions, had a standard deviation of ±60 per cent. Within a single experiment, however, relatively small differences among various experimental treatments are statistically significant, and since several hundreds of uniform explants can be taken from a single root, this does not in practice become a limiting factor for even the largest of experiments that are feasible.

Although all carrot-root explants give a marked response to many complex natural extracts such as whole coconut milk, the variability in their growth response becomes much more pronounced when purified fractions or pure compounds furnish the stimulus to growth. Even relatively crude fractions from coconut milk may induce growth responses, the magnitude of which are dependent upon unknown and inherent properties of the individual root from which the explants are drawn. Thus, explants from a given carrot root may be highly responsive to one fraction of coconut milk and only slightly responsive to another fraction, while for explants from another root the response to the same two fractions may be reversed. In such cases, the recombination of the coconut milk fractions almost invariably increases the growth response of explants from *both* roots, and the addition of casein hydrolysate further increases the response to the individual fractions as well as to recombined mixtures (Steward and Shantz, 1954).

The variation in the response to such growth factors of explants from different carrot roots becomes even greater when highly purified growth factors or pure compounds are tested. Compound A, a growth-stimulating substance originally isolated from coconut milk (Shantz and Steward, 1952) and later identified as 1,3-diphenylurea (Shantz and Steward, 1955) induced a wide range of growth responses when tested on explants from 12 different carrot roots (Steward and Shantz, 1954). These responses ranged from 3 cases in which no significant effect was observed to 4 cases in which the growth response was equivalent to that obtained by the addition of whole coconut milk. In all cases, however, the highly significant effect on the growth could be attributed to the diphenylurea only if casein hydrolysate was also present as a supplement to the basal nutrient medium.

In a study of the responses of carrot-phloem explants to indoleacetic acid, casein hydrolysate, and coconut milk, generally similar variations in response among explants from different roots were observed by Caplin (1956).

It has previously been suggested (e.g. Steward and Shantz, 1954, discussion section) that difficulties which arise from the inherent variation among carrot roots might be obviated by the use of clonal culture tissue. The use of such clonal cultures, however, imposes two limitations which detract from the advantages it might otherwise have. First, the removal of uniform, small explants to subculture such tissues presents many technical difficulties, and when the variability in the growth of such subcultures is compared with the variability of explants from the intact carrot root, it is found that the latter are much more suitable for the quantitative comparisons of growth responses. Second, the use of clonal tissue, by the very nature of such material, would exclude from the study many of the wide range of growth responses given by the direct explants from carrot roots, from which much may be learned.

THE EFFECTS OF NITROGENOUS SUPPLEMENTS TO MEDIA

When added to an otherwise complete nutrient medium, whole coconut milk exerts a strong growth-promoting power upon cultures of certain plant tissues (Caplin and Steward, 1948). This effect has been shown to be due to the interaction of a number of stimulatory substances which fall into three categories. One of these categories comprises a group of highly specific but as yet incompletely identified cell division-inducing compounds which are active in very small amounts. Three such compounds have been isolated in pure form (Shantz and Steward, 1952), of which one has been identified as 1,3-diphenylurea (Shantz and Steward, 1955). Other substances which produce similar effects include leucoanthocyanins and an indole acetic acid arabinose compound (Steward and Shantz, 1959). The second category of growth-promoting substances is non-specific in that its effect may be duplicated by, and is no doubt due to, free amino-acids such as those which occur in the coconut milk. This latter, non-specific fraction can, therefore, be replaced to a large extent by prescribed mixtures of individual amino-acids or by mixtures from such general sources as casein hydrolysate. The substances that induce cell proliferation, which are mentioned above, usually exhibit their best growth-promoting effect only when supplied in the presence of casein hydrolysate or similar mixtures of amino-acids. The third category comprises substances, other than known sugars, which are contained in a non-ionic fraction which is not absorbed on resins or charcoal, but which is nevertheless necessary for the cell division response (Shantz, Pollard and Steward, 1958).

Effect of casein hydrolysate on the growth of carrot-phloem explants

It was first observed in 1950 that casein hydrolysate could act as a co-factor for certain growth-promoting fractions obtained from coconut milk. It was also observed that, in the great majority of cases, the combination of coconut milk and casein hydrolysate induced a higher rate of growth than coconut milk alone, indicating that the amino-acids of whole coconut milk are present, either in quantity or in kind, somewhat below the optimal requirement of carrot tissue. These observations led, therefore, to a modification of the bio-assay procedure whereby all extracts, fractions, isolates, and test substances which were being assayed for their growth-promoting effect were tested in the basal medium and also in basal medium to which 200 p.p.m. of enzymatic casein hydrolysate had been added.

This has entailed the use of four different control media within each growth experiment, these being (1) the basal nutrient medium which is a modification of that of White, as described by Steward and Caplin (1948); (2) the basal medium to which has been added 200 p.p.m. of enzymatic casein hydrolysate; (3) the basal medium containing 10 per cent. by volume of whole coconut milk; and (4) the basal medium containing both coconut milk and casein hydrolysate. The average growth responses of many thousands of carrot-root explants subjected to these four treatments are given in Table 1.

TABLE 1

Effect of Casein Hydrolysate and Coconut Milk on the Growth of Carrot Phloem Explants

Initial weight of explants approximately 2·6 mgm. Average growth period (Group I) = 15 days, (Group II) = 19 days. Temperature 21° C., and the general conditions those described by Caplin and Steward (1949).

				Average final fresh weight of cultures grown in various control media			
Group	Period covered	Total no. of carrot roots	Approx. total no. of explants	Basal medium	Basal plus casein hydrolysate	Basal plus coconut milk	Basal plus casein hydrolysate plus coconut milk
I	1950–3	192	4,000	5·7 mgm.	19·8 mgm.	81·2 mgm.	105·2 mgm.
II	1954–7	255	10,000	7·9 mgm.	18·9 mgm.	95·6 mgm.	116·9 mgm.

From the data in Table 1 it can be seen that the addition of casein hydrolysate produces about a threefold increase in the relatively small amount of growth which is obtained in the basal medium alone, and gives a further increment of approximately 25 per cent. to the very much larger response attributable to the basal medium plus coconut milk. Even though the range of variability in growth responses among explants from different carrot roots is quite large, the increments resulting from the addition of casein hydrolysate are of extremely high statistical significance because of the very large number of cultures represented in the average weight figures. For group I in Table 1, the calculated probabilities that the increased growth resulting from the addition of casein hydrolysate could be due to chance variations were of the order of 10^{-7} (Shantz, 1954). The statistical distributions of these growth responses for group I have already been described (Steward and Shantz, 1954; Shantz, 1954).

Effect of casein hydrolysate on the growth of potato-tuber tissue cultures

Tissue from the potato tuber, on a coconut-milk medium, differs from carrot tissue in that an additional supplement of 2,4-D, or a similar growth-regulating compound, is required as a synergist, neither the 2,4-D nor the coconut milk alone being capable of stimulating maximum growth. This synergistic effect was first demonstrated by Steward and Caplin (1951), and the rather specific requirements of this system have since been extensively used to correlate the chemical structure of the 2,4-D-like moiety with the magnitude of the growth response (Shantz, *et al.*, 1955). It was, therefore, of interest to determine whether or not the potato-tuber tissue in culture also responded to casein hydrolysate in the same manner as carrot tissue.

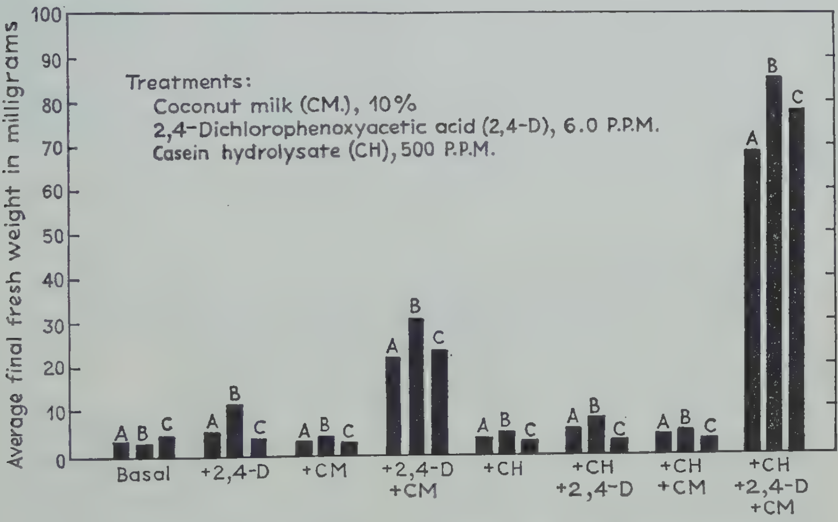

FIG. 1. Synergistic interactions of coconut milk, 2,4-D, and casein hydrolysate in stimulating the growth of 3-mgm. potato-tuber-tissue explants. Each treatment shows the average growth response of 5 replicate tissue cultures from each of 3 different tubers during a 26-day period.

Three such experiments were carried out, two of them concurrently, using explants from three different potato tubers. These were grown in the basal medium alone and in media to which were added supplements of coconut milk, casein hydrolysate, and 2,4-D. These three supplements were added individually and in all possible combinations. The average growth of the cultures under each treatment for each of the three experiments (A, B, and C) is shown in Fig. 1.

Except for a slight stimulation of explants from tuber B by 2,4-D alone, no significant response to any of the individual supplements occurred. Although the expected responses to 2,4-D and coconut milk were obtained, double combinations of casein hydrolysate with either 2,4-D or coconut milk were ineffective. However, when *all three* supplements were combined, a much greater growth occurred in every case, this being about three times that due to the active combination of coconut milk and 2,4-D. It is, therefore, apparent that casein hydrolysate induces a markedly increased growth when it is added as a further supplement to the medium which contains the rather specialized requirements of potato-tuber-tissue culture but, it should be noted, the casein hydrolysate is only effective when it is superimposed upon the conditions which cause the growth induction *per se.*

Comparison of casein hydrolysate and a mixture of amino-acids as nitrogenous supplements

To demonstrate that the effect of casein hydrolysate was, in fact, due to its free amino-acid content, a comparison was made between casein hydrolysate and a mixture of known amino-acids made up in the proportions that they have been shown to occur in casein hydrolysate, as recorded by Block and Bolling (1951). For this purpose, explants were taken from two carrot roots which, by prior test, were known to give a marked added growth response when casein hydrolysate was added to the medium containing coconut milk. The data from this experiment are shown in Table 2. To facilitate comparisons, the average final fresh weights for each treatment have been calculated relative to a value of 100 for the growth in the basal medium plus coconut milk.

TABLE 2

Effect of a Mixture of the known Amino-acids of Casein Hydrolysate Compared with the Effect of Enzymic Casein Hydrolysate on the Growth of Carrot-Phloem Explants

Each figure is the average of 4 replicate cultures

Treatment	Relative growth Carrot A	Relative growth Carrot B
Basal medium	15·0	10·4
Basal+Casein hydrolysate	15·8	20·7
Basal+Amino-acid mixture	15·6	17·0
Basal+Coconut milk	100·0	100·0
Basal+Coconut milk+casein hydrolysate	263·8	194·5
Basal+Coconut milk+amino-acid mixture	283·0	215·0

It is clear from Table 2 that the additive effect of coconut milk combined with casein hydrolysate was reproduced by the combination of coconut milk with the mixture of known amino-acids in the case of explants from both carrots. In the absence of coconut milk, explants from carrot A gave no response to the nitrogenous supplements, while those from carrot B gave a relatively small response. This and other similar experiments show that the casein hydrolysate effect can be reproduced by the use of a mixture of known amino-acids.

Ammonium chloride as a nitrogenous supplement for carrot-tissue cultures

The possibility arose that the tissue cultures may not require even amino-acids for their optimal growth but that they may merely require nitrogen in a reduced form. This was investigated by comparing the effectiveness of ammonium chloride with that of casein hydrolysate in stimulating the growth of explants from several different carrot roots, both in the presence and absence of coconut milk. In these experiments, casein hydrolysate was tested at a concentration of 200 p.p.m., while ammonium chloride was used at a concentration of 100 p.p.m.: this provides comparable levels of added nitrogen. The average growth for each treatment, relative to growth in the presence of coconut milk as controls to which a value of 100 was assigned, is given in Table 3. Each figure represents the average final fresh weight of 5 replicate cultures allowed to grow for 20 days from original explants which weighed 2·6 mgm. each.

Where the interaction of the nitrogenous supplements with coconut milk are compared (Table 3, treatments 4, 5, and 6), it can be seen that ammonium chloride has, in general, the same synergistic effect with coconut milk as has casein hydrolysate. The two extreme examples are in the explants from carrot F and carrot G. Carrot F gave a tremendous response to both nitrogenous additives as compared to the effect of coconut milk alone, and in the case of carrot G no significant growth response was observed with *either* supplement above that induced by coconut milk alone. In treatments 1, 2, and 3, however, where the supplements were added to the basal medium in the absence of coconut milk, a different effect was observed. In all cases, the addition of casein hydrolysate brought about the relatively small, though typically variable response, associated with this supplement as demonstrated previously by the average weight figures in Table 1. Ammonium chloride, on the other hand, *had no significant effect on the growth of explants from any of the seven carrot roots when added to the basal medium alone.*

TABLE 3

Comparison of Casein Hydrolysate and Ammonium Chloride as Nitrogenous Supplements for Carrot-tissue Cultures, using Explants from Seven different Carrot Roots

Treatment	Growth response relative to coconut-milk controls						
Carrot	A	B	C	D	E	F	G
1. Basal medium only	2·9	5·7	10·2	7·8	15·0	15·9	6·8
2. Basal + casein hydrolysate	9·5	22·8	20·4	18·7	45·3	39·7	21·1
3. Basal + ammonium chloride	2·4	6·1	8·1	6·3	11·4	17·3	5·7
4. Basal + coconut milk	100·0	100·0	100·0	100·0	100·0	100·0	100·0
5. Basal + coconut milk + casein hydrolysate	128·8	123·0	129·2	129·1	197·0	397·0	101·9
6. Basal + coconut milk + ammonium chloride	118·3	131·3	115·1	169·6	136·8	507·0	104·3

It would appear, therefore, that reduced inorganic nitrogen, in the form of ammonium chloride, can act as a supplement to whole coconut milk in the same manner as casein hydrolysate, but when this is supplied to the basal medium *alone*, it fails to produce the smaller growth response which is then attributable to casein hydrolysate.

The effect of varying proportions of nitrate and ammonium nitrogen, with and without casein hydrolysate, on the growth of carrot-phloem explants

In view of the interactions between ammonium nitrogen and coconut milk, as demonstrated in Table 3, the growth response was tested of carrot explants exposed to a combination of natural growth-promoting extract plus various mixtures of inorganic nitrogen, with and without casein hydrolysate. The inorganic nitrogen was supplied as mixtures of ammonium and nitrate ions in various ratios but at a constant total inorganic nitrogen level. Amino-acid nitrogen was supplied in the form of casein hydrolysate at the usual level of 200 p.p.m., calculated to furnish approximately 32 p.p.m. of nitrogen.

Whole coconut milk contains an appreciable quantity of free amino-acids and gives an optimal growth response at a fairly high concentration (10 per cent. by volume, or approximately 6,000 p.p.m. of dry weight). In the present experiment, therefore, the growth stimulus was provided by a concentrate of the liquid endosperm from immature fruits of *Aesculus woerlitzenis*. As has been previously reported (Steward and Shantz, 1955), this material is about 100 times as potent as whole coconut milk in stimulating the growth of carrot-tissue cultures. In addition, the extract had been partially refined by removal of protein, inorganic salts, and the bulk of the free amino-acids. At a concentration of only 40 p.p.m., in combination with casein hydrolysate, this preparation had previously been shown to be approximately equal to 10 per cent. of whole coconut milk in its growth-promoting power. Since the *Aesculus* concentrate gave only a very slight positive reaction with ninhydrin, it did not contain appreciable amounts of amino organic nitrogen.

The basal nutrient medium was prepared in the usual manner except that all nitrogen, normally supplied in the form of $Ca(NO_3)_2$, was omitted. The inorganic nitrogen supplements were added separately, in the form of either $Ca(NO_3)_2$ or NH_4Cl, to give a constant level of total inorganic nitrogen. The calcium level was maintained at its normal value in all treatments by the further addition of $CaCl_2$ where necessary. Besides the treatments in which the tissue received no nitrogen, nitrogen only as nitrate, and nitrogen only as ammonium, there were treatments in which the tissue received mixtures of the two inorganic forms of nitrogen in the proportions of $NO_3:NH_4$ of 0·9, 0·5, and 0·1. The growth responses to these various nitrogen supplements, with and without added casein hydrolysate, are shown in Table 4.

As shown by the data in Table 4, the explants from all three carrot roots responded in a qualitatively similar manner, although there were some quantitative differences among them, as shown by the total growth under the various treatments.

With no nitrogen at all (treatment 1) growth was relatively slight, though more than might be expected. The nitrogen source for this amount of growth was obviously the organic material stored in the original explants plus the traces of nitrogen added with the *Aesculus* endosperm supplement.

With the addition of nitrate in treatment 2, which is equivalent to the

TABLE 4

Effect of Nitrate-N, Ammonium-N, and Amino-acid-N on the growth of Phloem Explants from Three Carrot Roots in Modified White's Basal Nutrient Medium containing 40 p.p.m. of Aesculus *Liquid Endosperm Concentrate*

Original weight of explants = 2·6 mgm. Growth period = 28 days. Each figure for each treatment represents the average final fresh weight of 9 replicate cultures

	Concentration of nitrogen in p.p.m.			Average final fresh weight of cultures		
Treatment	As NO_3^-	As NH_4^+	As casein hydrolysate	Carrot A mgm.	Carrot B mgm.	Carrot C mgm.
1	0	0	0	33·4	20·4	46·2
2	32·0	0	0	99·2	81·6	96·1
3	28·8	3·2	0	137·6	95·9	122·7
4	16·0	16·0	0	98·6	69·7	104·6
5	3·2	28·8	0	30·4	24·7	34·1
6	0	32·0	0	17·7	13·8	16·6
7	0	0	32	82·7	61·2	82·5
8	32·0	0	32	127·0	111·5	146·0
9	28·8	3·2	32	149·1	129·5	150·0
10	16·0	16·0	32	103·5	89·9	91·8
11	3·2	28·8	32	51·4	52·6	52·4
12	0	32·0	32	31·0	49·9	37·7

regular basal medium as normally used, a predictable and large increase in growth occurred. A significant further growth increase resulted in all three cases by the substitution of ammonium nitrogen for 10 per cent. of the nitrate nitrogen (treatment 3). However, with increasingly larger fractions of the total nitrogen in the form of ammonium ion (treatments 4 and 5), growth was progressively depressed, and when all the nitrogen was supplied as ammonium ion (treatment 6) there was an inhibition of growth to a point where it was significantly less than that in the nitrogen-free medium.

When casein hydrolysate was furnished (treatments 7–12) in addition to the other supplements (treatments 1 to 6 respectively) an increased response occurred in all cases. Casein hydrolysate alone (treatment 7) was not quite as effective as nitrate alone (treatment 2) but the two were quite stimulatory when combined (treatment 8). The most effective treatment, however, was the one in which this combination was used with 10 per cent. of the nitrate nitrogen replaced by ammonium nitrogen (treatment 9). Further increases in the percentage of ammonium nitrogen (treatments 10–12) had an inhibitory effect on growth, although this inhibition was not as severe as in the analogous cases where casein hydrolysate was omitted (treatments 4–6).

It therefore seems quite evident, since comparable results were obtained in experiments using explants from three different carrot roots, that no single form of nitrogen provides a satisfactory supply for optimal nutrition of these tissue cultures. The combination of nitrate and casein hydrolysate (which in turn is replaceable by known amino-acids, see Table 2) is more effective than either supplement alone, and a further increase in growth response occurs upon the substitution of a relatively small amount of ammonium nitrogen for part of the nitrate nitrogen. Increasing the ratio of ammonium to nitrate, while maintaining a constant level of inorganic nitrogen, results in depressed growth. This decreased response is presumably due to either the increased ammonium/nitrate ratio or the diminished total nitrate level rather than the mere increase in total ammonium content, since no such inhibition occurred in the experiments described in Table 3 where ammonium chloride was used in relatively high concentration but where the nitrate concentration was also kept at the level which is normally used.

Urea and allantoin as nitrogen sources for the nutrition of carrot- and potato-tissue cultures

In a manner similar to the experiments described in Table 4, the efficacy of urea and allantoin as nitrogen sources for the nutrition of carrot- and potato-tissue cultures was studied. The nitrogenous supplements were added to a nitrogen-free basal medium (cf. Table 4) which contained an essentially nitrogen-free concentrate of *Aesculus* liquid endosperm as the growth stimulant. In the case of the potato-tissue cultures, the further addition of 2,4-D was required to induce growth. The average fresh weights of the cultures after growth under the various conditions of nitrogen supply are given in Table 5.

TABLE 5

Growth of Tissue Cultures from Explants of Carrot-root Phloem and from Potato Tuber, using Urea and Allantoin as Sources of Nitrogen

A nitrogen-free basal medium containing 40 p.p.m. of *Aesculus* liquid endosperm concentrate was used, and in the case of the potato-tissue cultures an additional supplement of 2,4-dichlorophenoxyacetic acid at 2·0 p.p.m. was furnished. Original weight of explants = 2·6 mgm. Growth periods = 24 days (carrot cultures) and 35 days (potato cultures). Each figure for each treatment represents the average final fresh weight of 12 replicate cultures.

	Nitrogen supplement as p.p.m. of N in form of			Average final fresh weight of cultures		
Treatment	Nitrate	Urea	Allantoin	Carrot A mgm.	Carrot B mgm.	Potato mgm.
1	0	0	0	19·9	21·9	8·1
2	32	0	0	32·4	43·5	24·7
3	0	32	0	57·5	76·4	10·0
4	32	32	0	58·8	79·5	25·6
5	0	0	32	34·1	39·4	26·1
6	32	0	32	54·8	71·8	50·3

The cultured explants from both carrot roots responded in a qualitatively similar manner. The response to allantoin was about the same as the response to nitrate in each case, but the response to urea was significantly better than to either of the other supplements (NO_3 or allantoin). Urea plus nitrate was no better than urea alone, but nitrate plus allantoin was the equivalent of urea. It appears that in the case of carrot tissue under the conditions of this experiment, urea is able to furnish a more complete type of nitrogen nutrition that is equivalent to that furnished by allantoin and nitrate in combination, but which can be supplied only in part by nitrate or allantoin singly.

In the case of potato tissue, a somewhat different type of response occurs. Here, urea alone was without effect, and urea plus nitrate was no better than nitrate alone. Allantoin alone, however, was the equivalent of nitrate, while allantoin and nitrate were complementary to each other and doubled the growth response when they were used in combination.

It again appears, therefore, that inorganic nitrate is able to furnish *only part of the optimal nitrogen* requirements for both carrot- and potato-tissue cultures. In both types of tissue, allantoin can supply at least a large part of the organic nitrogen requirements, with the combination of nitrate and allantoin being superior to either supplement singly. The two tissues differ markedly, however, with respect to their utilization of urea, which appears to satisfy both the organic *and* the inorganic nitrogen requirements of carrot tissue but is completely inadequate as a source of nitrogen for the potato-tuber tissue, which grows better on a combination of nitrate and allantoin.

The effect of tryptophan as a supplement to coconut milk

Early experiments on the addition of casein hydrolysate as a growth-promoting supplement to the basal medium showed that enzymically hydrolysed casein was slightly superior to the acid-hydrolysed material. Since tryptophan does not survive the acid treatment, the latter preparation was also tested with the further addition of tryptophan at a concentration of 5 p.p.m. in the medium, but no significant effect due to tryptophan was observed upon the growth of the cultures (Shantz, 1954).

More recently, in the course of another investigation, tryptophan was added to the culture media at a considerably higher concentration (50 p.p.m.). This was done in two ways: tryptophan in solution was added directly to the medium which was then sterilized by autoclaving in the usual manner (30 minutes at 15 lb. steam pressure), and a solution of tryptophan was sterilized separately by filtration before its addition to the previously autoclaved medium. These tryptophan solutions were added to the standard basal medium as well as to basal medium containing 10 per cent. of whole coconut milk. All treatments were repeated with the further addition of casein hydrolysate, and tested on explants from two different carrot roots. The average final fresh weights of the cultures after a growth period of 18 days are given in Table 6.

From Table 6 it is clear that a high level of tryptophan had only a slight effect upon growth in the basal medium, whether or not casein hydrolysate was present (treatments 1–3). In the presence of coconut milk, however, the rapid growth stimulated by the coconut milk (treatment 4) was significantly increased by the further addition of tryptophan, but only when it was autoclaved in the medium (treatment 6). This addition of autoclaved tryptophan not only increased the growth response due to whole coconut milk alone, but

Table 6

Effects of Autoclaved and Unautoclaved Tryptophan at 50 p.p.m. on Cultures of Carrot-Phloem Explants Grown in Basal Medium with and without Coconut Milk and with and without Casein Hydrolysate

Each figure represents the average final fresh weight of nine replicate cultures for each treatment

		Average final fresh weight of cultures			
		Without casein hydrolysate		Plus casein hydrolysate	
Treatments	Carrot	A mgm.	B mgm.	A mgm.	B mgm.
1. Basal medium		5·2	5·3	7·6	6·7
2. Basal+tryptophan (unautoclaved)		8·9	5·5	9·1	6·6
3. Basal+tryptophan (autoclaved)		7·5	6·1	14·6	6·4
4. Basal+coconut milk		44·2	78·1	63·6	105·5
5. Basal+C.M.+tryptophan (unautoclaved)		53·6	68·0	74·6	99·4
6. Basal+C.M.+tryptophan (autoclaved)		71·8	101·2	87·3	120·3

also enhanced in a similar manner the larger growth response induced by the combination of coconut milk and casein hydrolysate (cf. treatments 4 and 6, with and without casein hydrolysate). When the filter-sterilized tryptophan was added to the previously autoclaved medium, however, it had a much smaller growth-promoting effect on explants from carrot A and was slightly inhibitory to explants from carrot B. These results are somewhat similar to those of Roberts and Street (1955) who observed that autoclaved tryptophan had growth-stimulating activity for cultures of excised rye roots, but was ineffective when added aseptically to previously autoclaved medium. Their experiments also indicated that the activity of autoclaved tryptophan was not due to the formation of indoleacetic acid or indoleacetonitrile. It thus seems doubtful in the present experiments that tryptophan is acting as a source of nitrogen, but it appears rather that some substance arising from the heat treatment of tryptophan is acting as a more specific stimulus to cell proliferation.

Discussion

An essential feature of the medium which meets fully the requirements of actively proliferating tissue in culture is a source of nitrogen which does not limit the growth. The nature and concentration of the optimum nitrogen source can only be determined when the tissue is able to express its maximum ability to grow by cell division, and this pre-supposes that the cell division growth factors of coconut milk, *Aesculus* endosperm, or some equivalent fluid are present in non-limiting concentrations. Under these conditions it is evident that neither carrot cultures, nor potato-tuber-tissue cultures maintain their maximum growth on nitrate alone: these cells are, therefore, in a measure heterotrophic for nitrogen.

Earlier studies by Riker and Gutsche (1946; 1948) showed that when single amino-acids were used as nitrogen sources for the growth of sunflower callus-tissue cultures in a nitrate-free medium, only a few (alanine, glycine, arginine, asparagine, and aspartic acid) permitted growth to take place, while 11 others were inhibitory. All were inhibitory in a complete medium containing nitrate. Good growth was obtained with 0·08 per cent. casein hydrolysate in a nitrate-free medium, but in the presence of nitrate, casein hydrolysate was inhibitory, especially in concentrations higher than 0·3 per cent. These concentrations, however, are all higher than the 0·02 to 0·05 per cent. of casein hydrolysate found in the present study to be optimal for the growth of carrot-tissue cultures in the presence of coconut milk.

The use of various organic nitrogen compounds to replace or supplement inorganic nitrate in the culture of Jerusalem artichoke tissue has been studied by Nitsch and Nitsch (1957). In the presence of nitrate, a low concentration of enzymatic casein hydrolysate (2 mM./l. of N) was definitely stimulatory to growth, and more so than an equivalent amount of acid-hydrolyzed casein. At higher concentrations (10–20 mM./l.) in the absence of nitrate, the acid hydrolysate was found to be a satisfactory source of nitrogen, while the enzymatic hydrolysate inhibited growth completely. Again, however, these latter concentrations are much higher than those found in the present study to be optimal for the nutrition of carrot-tissue-cultures growth in the presence of coconut milk.

These same workers found that, among a number of compounds tested as sole sources of nitrogen, only α-alanine, γ-aminobutyric acid, glutamine, and glutamic acid were equivalent or superior to nitrate, while glycine, arginine, asparagine, and allantoin supported growth but were somewhat inferior to nitrate.

Paris and Duhamet (1953) have stated that the growth-promoting effect of coconut milk on salsify crown-gall tissue could be duplicated by a mixture of vitamins and amino-acids, although the vitamin mixture alone or the amino-acid mixture alone had but slight effect. This response could not be obtained with cultures of carrot tissue (Shantz, 1954). Paris and Rietsema (1953) also studied the nitrogen nutrition of *Datura* embryos on a synthetic medium. They observed that individual amino-acids were generally ineffective although growth was promoted, especially in the smaller, more immature embryos, by either a mixture of amino-acids or by casein hydrolysate. Sanders and Burkholder (1948), also studying the culture of *Datura* embryos, found that the addition of 0·01 per cent. to 0·08 per cent. of casein hydrolysate markedly improved both growth and embryonic differentiation. This effect could be duplicated by a mixture of 20 amino-acids approximating the composition of casein hydrolysate, but lesser mixtures gave considerably less growth. Individual amino-acids were ineffective, although they had a variety of effects on morphological development.

The nitrogenous requirements for the growth of rapidly proliferating tissue cultures and the interpretation of the effects of such fluids as coconut milk in the stimulation of that growth now need to be considered against the entire background of knowledge which is available of these cells and of their metabolism.

First, it is now clear that the role of the fluids, such as coconut milk, which normally nourish immature embryos but which can also restore some otherwise differentiated and relatively mature cells to the actively dividing state, is complex and comprises effects of different kinds. There are the highly specific catalytic effects of the growth factors proper, for these act at very low concentrations, and they produce in the cells a variety of morphogenetic effects (Steward, Mapes, and Smith, 1958). There are also effects of a more strictly nutritional kind, which are non-specific, in the sense that the coconut milk or other natural fluids are *in these respects* replaceable by a variety of known substances. Such non-specific, nutritional effects can be seen when the best means of furnishing the nitrogenous requirements of the tissue are sought, for this necessitates that nitrate be supplemented with casein hydrolysate or some other form of reduced nitrogen. However, it is apparent and should be recognized that even the best exogenous sources of nitrogen are not *alone* competent to induce rapid growth in the otherwise resting cells. It is necessary to make this point, because those who first sought to designate the growth-promoting factors of coconut milk and failed to find any unique qualities or novel substances responsible for this effect, took refuge in the claim that its virtues lay only in some subtle combination of already well-known nutrients (Van Overbeek, Siu, and Haagen-Smit, 1944; Paris and Duhamet, 1953).

Therefore, although coconut milk does in part satisfy certain nitrogenous requirements of cultured carrot cells, over and above those that can be satisfied by nitrate, it is far from an optimum source in this respect. The needs of the tissue for this optimum nitrogenous supply are created by those constituents of coconut milk which cause the cells to grow rapidly. Thus the nitrogenous need of the tissue are the *result* of the stimulus to grow, and the nitrogenous nutrients in the coconut milk cannot be regarded as its sole cause.

The search for a fully competent source of nitrogen for the rapidly growing cells of the carrot discloses that nitrate alone only supports the growth to a limited extent. To be more effective, ammonia must also be present with the constituents of coconut milk, where it can profitably replace nitrate in a mixture of nitrate to ammonia in the ratio of 9:1. The ammonia obviously acts in association with some organic moiety of the coconut milk, either before or after they are absorbed by the cells. The obviously growth-stimulating effects which can now be attributed to casein hydrolysate, which is replaceable by known amino-acids supplied in nutritional rather than catalytic amounts, are also provided by ammonia as a source of reduced nitrogen when the ammonia is furnished in association with the organic complex of the coconut milk (i.e. other than the cell division factors *per se*). But the most important conclusion is that the most competent single nitrogenous nutrients for the dividing carrot cells is urea. Urea is the only substance that seems able to act in lieu of the most effective combinations of nitrate or ammonia and organic nitrogen (amino-acid). This requirement is not for ureide-N in general, for allantoin will not wholly replace urea, though it will do so in combination with nitrate.

The effectiveness of urea in the support of growth has been investigated primarily with relation to nitrogen-heterotrophic organisms (*Neurospora* in particular) and with respect to its role in the arginine-ornithine cycle. Complex interactions have been noted in this area also. For example, Srb (1950) showed that an arginine-requiring mutant which would not grow on urea grew better on arginine plus urea than on arginine alone. It grew better still with arginine and guanylic acid, and slightly better again on arginine, urea, and guanylic acid. This suggests that urea supplies part of the growth requirements that may otherwise be satisfied by purines or pyrimidines.

It is clear that the cultured cells of carrot, pushed to the limit of their growth, also become in a measure heterotrophic because their requirements are not fully satisfied by nitrate. The now rather general agricultural use of urea as a source of nitrogen for crops may indicate a greater measure of adaptability to a heterotrophic type of nutrition than would otherwise be supposed for higher plants.

It is now necessary to consider the results of the exogenous supply of nitrogen along with what is known of the endogenous substrates that furnish nitrogen for growth and protein synthesis. It goes without saying that growth and protein synthesis proceed concomitantly. It is, however, abundantly clear that the free amino-acids of the cells do not alone furnish the carbon and nitrogen supply for this synthesis. Other studies of the protein and nitrogen metabolism and the respiration of the actively growing carrot cells have demonstrated the following points which are now relevant to this discussion:

In the dividing cells the soluble nitrogen is maintained at a low level in comparison with that which obtains in the resting cells. This suggests that nitrogen is drawn into protein almost as fast as it can be mobilized into the appropriate form in the cells. The compounds that remain relatively conspicuous in this scanty complement of soluble nitrogen are alanine, glutamine, and γ-aminobutyric acid (Steward, Thompson, and Pollard, 1958).

The tissue does not contain or store the amino-acids in the proportions required to form protein, so that the free amino-acids do not, simply, solely, and directly, act as the immediate substrates which furnish the carbon or the nitrogen for the synthesized protein. It is postulated that the carbon in the newly formed protein enters it more directly from exogenous sugar than from the amino-acids already present, whereas the latter, as products of protein breakdown, seem to furnish carbon for respiration more readily than might otherwise be supposed. The nitrogen for synthesis reaches the protein synthesizing surface in some readily usable form where it can condense with compounds derived from sugar to form protein. Glutamine seems to be important in furnishing this readily usable nitrogen, while its carbon framework may be respired away via the organic acid cycle (Steward and Street, 1946).

The exogenous supply of radioactively labelled substrates such as sugar, glutamine, and γ-aminobutyric acid has yielded results which are compatible with the ideas above. However, the protein of these dividing cells has a unique component, distinguished by its extreme stability and resistance to metabolism, which contains combined hydroxyproline formed *in situ* from proline. Although exogenous proline is very rapidly and readily incorporated into this protein, it is quite clear that the cells can, with any competent nitrogen and carbohydrate source, make enough proline so that this substance does not become a limiting factor unless blocked at the site of its formation or use as, for example, by hydroxyproline (Steward, Pollard, Patchett, and Witkop, 1958).

It is clear that there is some special peculiarity of urea in its relation to growing carrot cells, though not for potato cells. In the latter, any distinctive role as a nitrogenous supplement has only been seen for allantoin. The commonly accepted interpretation of the utilization of urea by plants is that it is split by urease into ammonia and carbon dioxide, and therefore functions merely as an alternative source of these substances. This is not so when urea is furnished to growing carrot cells (Pollard, Bollard, and Steward, 1957), for the paths of carbon are quite different when C^{14}-urea, or $C^{14}O_2$ and ammonia are alternatively supplied. Whereas the $C^{14}O_2$ rapidly and effectively enters the protein, the C^{14}-urea labels a variety of soluble compounds, not mainly amino-acids, which can be detected on radioautographs of chromatograms. These compounds are not those detected when carrot tissue is treated with C^{14}-sugar or C^{14}-glutamine (Steward, Bidwell, and Yemm, 1956). In the culture of potato tissue urea plays no distinctive role, but it will either enrich the pool of glutamine or remain largely as urea and ureide according to certain innate properties of the variety of tuber (Steward and Pollard, 1956). All this points to the fact, evident also from the results here described, that there is some feature of the urea molecule that makes it especially competent to act as a nitrogenous nutrient for rapidly growing carrot cells. In this relationship both the nitrogen and the carbon of urea must be involved.

More work will be necessary to trace the fate of urea in growing carrot cells and to explain its role as a nitrogen source. One unproven explanation is that urea may readily lead in the tissue to the carbamyl group, which may then participate in group transfer reactions. A spot in the position of ureido succinic acid (readily formed from urea and malic acid) was observed on chromatograms and radioautographs of extracts of tissue treated with C^{14}-urea, although identification needs support by evidence other than that of position alone.

Ample justification can be cited for the general idea that the efficacy of urea (or allantoin) in the special nutrition of these dividing cells should be sought in their relations to purine-pyrimidine metabolism, rather than amino-acid metabolism. It is true that until recently urea and allantoin have been thought of more as products of catabolism than as intermediates or precursors for synthesis, though the synthesis of allantoin from glyoxylic acid and urea, and reciprocal relations of allantoin and glutamine have been noted (Tracey, 1955).

The probable nature of the natural 'carbamyl-donor' in the conversion of ornithine to citrulline as carbamyl phosphate, and the finding that it can be formed from CO_2, NH_3, ATP, and Mg^{++}, all suggests that the efficacy of urea as a nitrogenous supplement may be due to its intervention in the reactions of carbamyl groups, particularly when the cell-division growth factors create a heavy demand for the fragments necessary to build nucleic acids. Certain now well-recognized paths make this plausible. These are the combination of CO_2, NH_3, and ATP or carbamyl phosphate with aspartic acid to give eventually, via ureidosuccinic and orotic acids, the pyrimidine uridine-5-phosphate. This links urea with the synthesis of important pyrimidines which might otherwise be limiting in the cells (cf. Ruhland, 1958, pp. 791 et seq.). Similar relationships, though not known to be reversible, exist between uracil and β-alanine via β-ureidopropionic acid (Ruhland, 1958, pp. 798–9).

Thus, granted that carrot tissue seems well able to synthesize protein from sugar and endogenous nitrogen sources even better than from amino-acids, it seems logical to seek the explanation of the need for reduced forms of nitrogen to supplement nitrate in some ultimate requirement which is not solely for amino-acids. Even when these are directly furnished, the nitrogen used for protein synthesis seems to be first drawn into the metabolic pool. The fact that urea seems to supply nitrogen in association with carbon in some particularly effective form points to its possible role in the synthesis of purines and pyrimidines and eventually of nucleic acids. It should be mentioned, however, that exogenous supplies of purine and pyrimidine nitrogen (as single compounds or in combination) have not yet been found effective in replacing the growth stimulus of the coconut milk for the carrot tissue.

Two amino-acids present in enzymic casein hydrolysate are in a somewhat special position: cystine, because of its ability to furnish amino-acid sulphur, and tryptophan, because of its possible relationship to indoleacetic acid. Although Gautheret (1942) habitually supplies cystine in his basal medium, this has not been done here. Since such a rapid growth of carrot tissue is obtained with coconut milk plus casein hydrolysate or urea, there seems to be little room left for a marked response to additional cystine. This presumably means that if supplied with appropriate sources of nitrogen and natural growth factors, the tissue can reduce inorganic sulphate at a rate sufficient to supply its own sulphur amino-acid requirements. Our earlier results on the addition of cystine are consistent with this view.

Tryptophan is present in enzymic casein hydrolysate, though it may be partially decomposed on autoclaving. Experiments using relatively high concentrations of tryptophan for the nutrition of carrot-tissue cultures have shown that tryptophan does indeed act synergistically with whole coconut milk. To achieve these responses, however, much higher levels than could be accounted for by the addition of casein hydrolysate are required, and it appears quite possible that some heat-degradation product of tryptophan is acting in very small amounts as a stimulant to cell division. Further work on this problem is in progress and will be reported elsewhere.

SUMMARY

In a study of the nitrogen nutrition of carrot phloem and potato-tuber-tissue cultures, stimulated into rapid proliferative growth by the liquid endosperms of coconut or *Aesculus*, the following observations have been made:

1. Nitrate alone is inadequate as a nitrogen source for the optimal growth of carrot and potato-tissue cultures under the stimulatory effect of coconut milk.

2. The addition of casein hydrolysate gives an increased response to coconut milk and similar fluids. In the absence of coconut milk, casein hydrolysate gives a much smaller and more variable response. It is replaceable by a mixture of amino-acids, but individual amino-acids are in general much less effective.

3. Ammonia-N alone does not stimulate any increase in growth (as does casein hydrolysate, to a limited extent), but allows an increased response to coconut milk in the same manner as does casein hydrolysate. It is most effective when used to replace 10 per cent. of the nitrate-N. A mixture of all three supplements (nitrate, ammonia, and casein hydrolysate) is superior to any one of them individually or in double combinations.

4. Urea is effective as a sole source of nitrogen for carrot tissue but does not support growth in potato tissue. Allantoin supports growth of both tissues but is more effective when combined with nitrate.

5. Tryptophan, when supplied at a relatively high concentration and autoclaved in the medium, acts synergistically with coconut milk to produce an increased growth response. It is considered more likely that some product of tryptophan is acting as a specific stimulant to cell proliferation rather than as a nitrogen supplement.

6. The possible metabolic roles of the various nitrogen supplements have been discussed, with special reference to urea.

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Experimental and Analytical Studies of Pteridophytes

XXXVIII. Some Observations on Spiral and Bijugate Phyllotaxis in *Dryopteris aristata* Druce

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With three Figures in the Text

ABSTRACT

In an attempt to assess some of the factors which determine leaf arrangement at the shoot apex, a study has been made of phyllotaxis in the fern *Dryopteris aristata*. It is shown that clockwise and counter-clockwise spirals occur with equal frequency both in field collections and in adventitious and experimentally induced buds which arise on the rhizomes and apices of plants grown in the laboratory. In addition to the two common spiral arrangements, a form with bijugate phyllotaxis has been noted. It is concluded that the three types of leaf arrangement are not under direct genetic control, but rather are dependent on circumstances of growth at the apex.

INTRODUCTION

IN plants with spirally arranged leaves, the direction of the genetic spiral, i.e. that spiral which passes through all the leaves in the order of their formation, can be either clockwise or counter-clockwise. As has long been known (Bravais and Bravais, 1837) the direction of the spiral on the branches of a plant is not necessarily the same as that on the main axis. Moreover, in *Lycopodium* the two apices resulting from a dichotomy may have spirals in opposite directions (Church, 1904). It is usually considered, in consequence, that the direction of the phyllotactic spiral is not directly controlled by genetic factors. Indeed, Richards (1948) considers that 'the phyllotaxis of an apex is dependent on circumstances and, if interfered with, may readily change from one system to another'. Raunkiaer (1919) has examined the direction of the spiral in plants of several species of angiosperms and in two species of mosses, but, on the whole, relatively few records of observations on a large number of specimens of a single species are available and, to the authors' knowledge, none on ferns. The following observations on *Dryopteris aristata* may therefore be of interest.

In addition to the two spiral forms, occasional apices of *Dryopteris* are found which cannot be relegated to the usual spiral system. Apart from a very few irregular apices, the phyllotaxis of these specimens was found to be bijugate; that is, the leaves are arranged in opposite pairs which diverge by an angle of less than 90°. In bijugate phyllotaxis two genetic spirals, each passing through one member of each pair, can be constructed (Bravais and

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Bravais, 1837; Church, 1904; Snow, 1951); the divergence angle along each spiral is equal to half the Fibonacci angle, at least in higher systems (Bravais and Bravais, 1837; Richards, 1948). The occurrence of bijugate phyllotaxis in *Dryopteris* is of particular interest for two reasons: firstly, because of the rarity of whorled leaf arrangements in ferns (Bower, 1923; Schoute, 1938), and secondly, because it offered an additional means for studying whether or not phyllotaxis at the fern apex was under direct genetic control.

MATERIALS AND METHODS

Rhizome tips were collected from various localities, the shoot apices and young primordia laid bare, and the plugs of tissue maintained in pans of damp peat in the laboratory (Wardlaw, 1944, 1947). In some specimens, buds were induced from the presumptive sites of leaf primordia by various systems of deep incisions. After a period of several months adventitious buds also formed on the older leaf bases. Leaf primordia were punctured with a sharpened steel sewing needle. Observations of phyllotaxis were made at the shoot apex, and accordingly the terms clockwise and counter-clockwise here refer to the direction of the spiral passing from younger to older leaf primordia, i.e. the converse of the genetic spiral.

OBSERVATIONS

(i) *Bijugate apices*

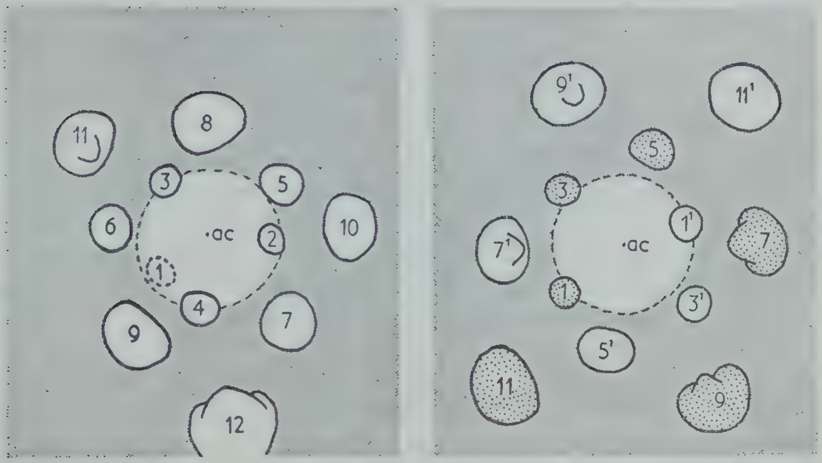
Spiral and bijugate apices of *D. aristata* may be compared in Figs. 1 and 2. It will be seen that in bijugate specimens the primordia are arranged in opposite pairs through which two genetic spirals can be constructed (Fig. 2, in which the primordia along one of the spirals are stippled). Drawings of 18 apices were made with a camera lucida, and the divergence angles between successive primordia along the two spirals measured. The average divergence angle for individual spirals ranged from 67° to 74°; for the 36 spirals measured the overall average was $70\frac{1}{2}°$. This angle thus closely approximates to half the Fibonacci angle (68·75°), as expected.

As Richards (1951, p. 530) has pointed out, in bijugate apices it is also possible to construct four spirals along which successive primordia diverge by the Fibonacci angle (Fig. 3). This can be seen more readily in *Dryopteris* than in other bijugate specimens, because of the small size of the leaf primordia relative to the shoot apex and the large number which can be viewed at one time.

(ii) *Field collections*

Over a period of several years the phyllotactic systems of *Dryopteris* apices which had been collected at all seasons of the year, and in several different localities, mainly in Cheshire, were recorded. Table 1 shows the number of apices placed in each of the three phyllotactic categories.

The nearly one to one proportion of the two spiral forms supports the view



FIGS. 1 and 2. Surface views of spiral and bijugate apices, respectively. In Fig. 2 the primordia belonging to one of the two genetic spirals are coarsely stippled. The sub-apical region is stippled; the approximate extent of the apical meristem is indicated with a broken line. *ac.* = apical cell. (× 18).

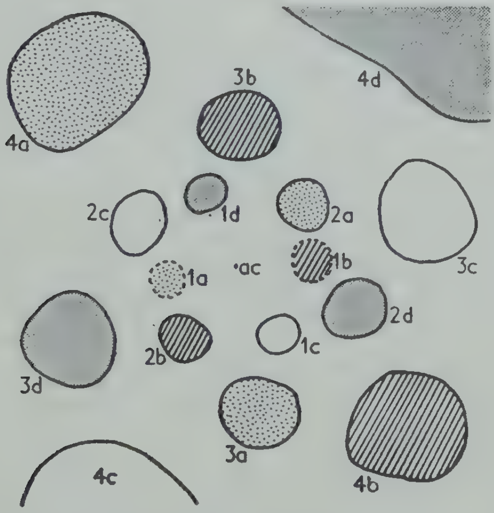


FIG. 3. Surface view of a bijugate apex, showing the possible four Fibonacci spirals (*a*, *b*, *c*, and *d*). *ac* = apical cell. (× 18).

that the direction of the spiral is randomly, and not genetically, determined. As can be seen, the bijugate apices comprise a substantial proportion, 8·6 per cent., of the total. Apices with phyllotaxis of this type were recorded from 26 of the 45 collections; they occurred in collections from four distinct

TABLE 1

Phyllotaxis of Apices from Various Localities

Spiral		Bijugate
Clockwise	Counter-clockwise	
473	492	91

localities in Cheshire, England, and from one in East Lothian, Scotland. Similar apices, not included in Table 1, have also been collected in Lancashire (Dasanayake, personal communication). Thus the distribution of plants with this type of phyllotaxis is not merely local. Although supporting the view that the two spiral forms occur at random, the evidence of Table 1 alone does not permit any conclusion on the possibility of genetic control of bijugate phyllotaxis in this species.

(iii) *Phyllotaxis of induced and adventitious buds*

If leaf arrangement in *Dryopteris* were genetically determined, it would be expected that the phyllotaxis of adventitious and experimentally induced buds would be correlated with the phyllotaxis of the main apex. Apices collected in October were grown in pans of peat in the laboratory for a period of 6 months, in the course of which a number of adventitious buds developed; in some specimens buds were induced from portions of the apex by a system of deep cuts, as previously described by Wardlaw (1949*a*). Table 2 shows the leaf arrangements of both the adventitious and experimentally induced buds which developed on rhizomes with either spiral or bijugate phyllotaxis. Since the phyllotaxis of such buds is often somewhat irregular at the outset, determination of the type of phyllotactic system was carried out only after several primordia had been formed.

From Table 2 it is evident that buds having any, or even all, of the three types of phyllotaxis may be produced on a particular rhizome, irrespective of the phyllotaxis of the original apex of that rhizome. In clear agreement with data from the field collections, the two spiral forms (*c.* 91 per cent.) distinctly predominate over the bijugate form (*c.* 9 per cent.), and the total number of clockwise and counter-clockwise forms is almost equal (33 to 36). Although the number observed is small, it may be pointed out that in those rhizomes with normal spiral phyllotaxis the total number of adventitious and induced buds with spirals in the same direction as the original apex (homodromous) was very nearly equal to the number possessing spirals in the reverse direction (antidromous), i.e. 20 and 21. It may be noted, also, that the phyllotaxis of the majority of adventitious buds formed on rhizomes with bijugate phyllotaxis was of the normal spiral type. The factors controlling this type of phyllotaxis in *Dryopteris* are evidently not genetic.

DISCUSSION

The observations reported here seem to indicate that, in *D. aristata*, there is no direct genetic control either of the direction of the phyllotactic spiral,

TABLE 2

Phyllotaxis of Adventitious and Induced Buds

		Number of buds		
Rhizome	Original apex	Clockwise	Counter-clockwise	Bijugate
1	Clockwise	5	6	—
2	,,	1	1	—
3	,,	4	7	2
4	,,	3	2	—
5	,,	2	—	—
6	Counter-clockwise	—	3	—
7	,,	1	2	—
8	,,	3	—	—
9	,,	1	—	—
10	Bijugate	2	2	1
11	,,	2	—	—
12	,,	1	1	—
13	,,	—	1	—
14	,,	4	7	1
15	,,	1	1	—
16	,,	2	3	1
17	,,	1	—	2
Total		33	36	7

or of the occurrence of spiral or bijugate phyllotaxis. In the field collections approximately equal numbers of apices with clockwise and counter-clockwise spirals were found; moreover, it was shown that the phyllotaxis of adventitious or induced buds was not correlated with that of the rhizome on which they were formed. This evidence from the ferns is in keeping with most observations relating to the statistical occurrence of clockwise and counter-clockwise spirals, and of homodromous and antidromous lateral shoots, as presented for a considerable number of flowering plants by Raunkiaer (1919) and Compton (1910; 1912–13). Natural populations of angiosperms regularly comprised equal numbers of right- and left-handed spirals, although Raunkiaer showed that in at least some species the direction of the spiral of lateral buds showed a high negative correlation with that of the main apex. For example, in 1,000 specimens of *Sarothamnus scoparius*, 96 per cent. of the lateral shoots were antidromous, and only 4 per cent. homodromous.

Other evidence from various experiments is compatible with the view that phyllotaxis is not rigidly determined by genetic factors but is influenced by a combination of factors which initiate particular systems. For example, apices with spiral phyllotaxis have been obtained from the two halves of split apices of decussate species (Snow and Snow, 1935; Snow, 1942); furthermore, the two apices thus formed sometimes possessed spirals in opposite directions (Snow and Snow, 1935, Fig. 6). In some cases the direction of the 'genetic spiral' has also been experimentally reversed by various treatments (Snow and Snow, 1931, 1933; Wardlaw, 1949*b*). In a considerable number of species with whorled phyllotaxis, Weisse (1894) found that experimentally induced adventitious buds usually showed spiral arrangements. Discussing their own and Weisse's experiments, Snow and Snow (1935) conclude that the difference between species with decussate and spiral phyllotaxis cannot be ascribed to intrinsic differences in the physiological properties of their apices, but probably depend on how the systems originate. The present observations on *Dryopteris* are in accord with Weisse's (1894) findings, and the conclusion of Snow and Snow (1935) may therefore be extended to include bijugy.

The occurrence of specimens with distinct phyllotactic systems in a single species is clearly of interest, particularly in view of the lack of contact between leaf primordia in ferns. Comparative studies of bijugate and spiral apices of *D. aristata*, with particular reference to growth, are in progress and will subsequently be reported elsewhere.

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The Structure and Development of the Reproductive Organs and Carposporophyte in two British Species of *Gelidium*

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With four Figures in the Text

ABSTRACT

The structure and development of the reproductive organs and carposporophyte are described for two British species of *Gelidium*, *G. latifolium* (Grev.) Born. & Thur. and *G. pulchellum* (Turn.) Kütz. The gonimoblast does not develop directly from the unchanged carpogonium, as was thought by Kylin, but a swollen multinucleate cell of irregular outline is formed first, either from the carpogonium alone, or by the fusion of the carpogonium and certain neighbouring cells; the gonimoblast develops from long, non-septate processes of this multinucleate cell. The significance of the fusion of the carpogonium and certain vegetative cells is discussed in relation to the various definitions of the auxiliary cell which have been proposed. It is concluded that an auxiliary cell does not occur in *Gelidium*.

INTRODUCTION

THE classification of the Florideae, as developed by Kylin, is based principally upon the structure and development of the carposporophyte. Of all the orders of the Florideae, least is known of the reproductive structures of the Gelidiaceae. Apart from occasional figures in taxonomic studies, the brief notes of Bornet and Thuret (1876) on the structure of the sexual plants and the 'cystocarp' of *Gelidium latifolium* and the more detailed account by Kylin (1928) of the structure of the sexual plants and the early stages in the development of the carposporophyte of *G. cartilagineum* are the only descriptions of the reproductive structures of species of *Gelidium*. Bornet and Thuret (1876) have described also the structure of the mature 'cystocarp' of *Pterocladia capillacea* [*Pt. pinnata* (Huds.) Papenf.], but nothing is known of the developmental stages. Previously regarded as a family of the Nemalionales, the Gelidiaceae was raised to the status of an order by Kylin (1923; 1928), because of the supposed absence of an auxiliary cell and the occurrence of tetrasporangium-bearing plants. This separation of the Gelidiaceae from the Nemalionales must be reconsidered in relation to the increased knowledge of that order obtained in recent years. For this, a detailed examination of the structure and development of the reproductive organs and carposporophyte is opportune.

The following account is based upon a detailed investigation of the two

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British species of *Gelidium*, *G. latifolium* (Grev.) Born. & Thur., and *G. pulchellum* (Turn.) Kütz. As in a previous paper (Dixon, 1958), the specific names advocated by Feldmann and Hamel (1936) are used pending a full taxonomic revision of the British species, varieties, and forms.

MATERIALS AND METHODS

Gelidium pulchellum and *G. latifolium* occur frequently in the British Isles, but carpogonial and 'cystocarpic' plants of both species are extremely rare. The collections used in this investigation are as follows:

G. pulchellum.

Inis Meadhon, Aran Islands, Eire, 18.viii.1943; Spiddal, Co. Galway, Eire, 11.i.1955; Venus Pool, Sark, Channel Islands, 16.viii.1955; Vazon Bay, Guernsey, Channel Islands, 10.viii.1955.

G. latifolium.

Whitestrand Bay, Co. Donegal, Eire, 17.ix.1943; Borth Saint, Holy Island, Anglesey, 27.x.1950; 11.x.1951; 17.ix.1952; Salthill, Co. Galway, Eire, 20.viii.1951; Prah Sands, Cornwall, 13.viii.1954; Porth-y-Garron, Holy Island, Anglesey, 20.ix.1951; 7.xii.1955; Mill Bay, Pembrokeshire, 22.ix.1956. Spermatangial plants of *G. latifolium* were collected at Borth Saint, Holy Island, Anglesey, 11.x.1951 and Mill Bay, Pembrokeshire, 22.ix.1956, but spermatangial plants of *G. pulchellum* have not been collected in the British Isles. Tetrasporic plants of both species are extremely common in all parts of the British Isles.

Several fixatives were used, but the best results were obtained with formalin-alcohol and formalin-acetic-alcohol. Microtome sectioning was used for the investigation of the gross anatomy, but the use of squash preparations was necessary for the detailed study of the development of the carposporophyte. For squash preparations, the material was softened in an acid-alcohol mixture and then squashed under an albumenized coverslip. Brazilin was used principally for staining the squash preparations, whilst Heidenhain's iron alum haematoxylin was employed chiefly for the serial sections.

THE DEVELOPMENT OF SPERMATANGIA

Spermatangial fronds of *G. latifolium* are very small and insignificant, so that without microscopical examination it is often impossible to distinguish them from the small sterile fronds which occur so frequently in the British Isles. From the frequency with which spermatia have been observed, attached to the trichogynes of *G. pulchellum*, it is probable that male plants of this species do occur in Great Britain, but that they have been overlooked. The apparent rarity of spermatangial material of *Gelidium* in the British Isles is the result of the insignificant appearance of the male plants.

The development of a spermatangial frond is similar to that of a sterile axis (Dixon, 1958). The spermatangia occur superficially as irregular patches on the main axes and lateral branches of the frond. The superficial cortical cells, that is, the apical cells of the lateral branches of limited growth, enlarge to form spermatangial mother cells. Each of these gives rise to two or three spermatangia, which divide transversely after their formation (Fig. 1*a*). As the thallus ages, the superficial layer of mucilage over the sorus increases in thickness and as a result mature spermatangial fronds may be recognized by the presence of a distinct colourless margin, three to four times the normal thickness of the mucilage. It is not clear whether spermatia are liberated from both the upper and lower cells formed by the transverse division of the spermatangium. After a spermatium has been liberated from the upper cell, the lower cell appears to remain intact for a considerable time.

The transverse division of the spermatangium immediately after its formation has been reported by Bornet and Thuret (1876), Kylin (1928), and Smith (1955) in various species of the genus. Newton (1931, Fig. 160*c*), on the other hand, figures the spermatangia as occurring in chains, but no evidence of this has been obtained, either by previous investigators, or during the present study.

The Carpogonium

The carpogonia of both *Gelidium pulchellum* and *G. latifolium* are formed in the apical regions of the short lateral axes which occur on each side of the principal axes in these species. Growth of these lateral axes appears to be inhibited by the development of the carpogonia, so that the resulting 'cystocarp' is always terminal, although a short 'beak' may be present occasionally. The development of a carpogonial axis is similar to that of a sterile axis (Dixon, 1958) except that the cortex is composed ultimately of cells which are larger, with more prominent nuclei, than the cortical cells of sterile axes. In addition, the secondary pit-connexions develop only in a lateral direction, so that a conspicuous plate of cells, one cell in depth, is formed.

The development of a carpogonium is initiated by the formation of a lobe on a cell of a lateral branch of limited growth, a short distance behind the apical cell of an axis. The lobe elongates rapidly and it is then cut off by a wall to form a sessile carpogonium although in some examples the wall does not develop and the carpogonium is intercalary. The carpogonium continues to elongate and the apical prolongation penetrates the surface of the thallus to form the trichogyne. There is a marked constriction at the surface of the thallus between the trichogyne and the basal part of the carpogonium. When mature, the uninucleate carpogonium is elongate, up to 30μ in length, triangular in outline and slightly curved, with a breadth of from $4{\cdot}0$ to $6{\cdot}5\mu$. The trichogyne is cylindrical and of variable size (Figs. 1*b*, *c*, and *d*).

The development of nutritive tissue does not commence until after the maturation of the carpogonium. A number of conical primordia are formed from and around the cell below that which gave rise to the carpogonium. These primordia segment to produce short, branched filaments of cubical cells which are inclined slightly towards the axial plate and which form an umbrella-like structure surrounding the parent cell. Because of its relatively late initiation, the nutritive tissue is not fully developed until after the development of the carposporophyte has commenced (Figs. 2*a* and *b*). The cells of the mature nutritive tissue have thin walls, large nuclei and deeply staining contents. During the early stages of the development of the carposporophyte, the cell contents of the nutritive tissue disappear gradually, although the empty cell-walls can be discerned for a considerable time.

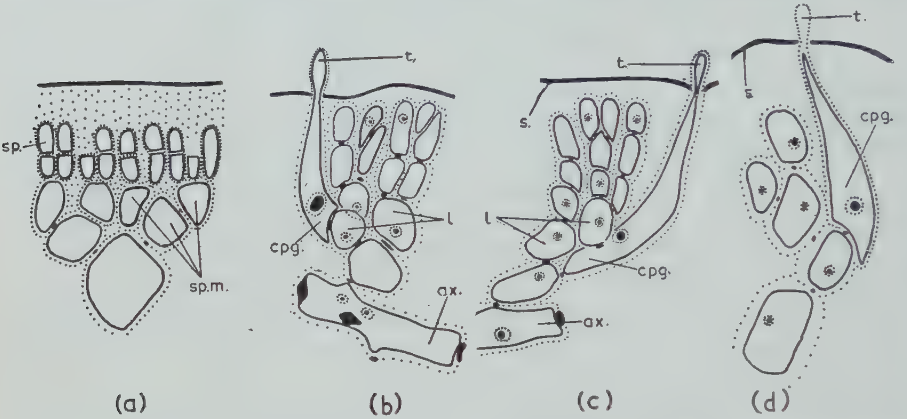

FIG. 1. (*a*), Section of spermatangial sorus; (*b*), (*c*), (*d*), the carpogonium in relation to the lateral branches of limited growth: (*a, c, d, G. latifolium; b, G. pulchellum*); (*ax.*, axial cell; *cpg.*, carpogonium; *l.*, lateral branches of limited growth; *s.*, surface of the thallus; *sp.*, spermatangium; *sp.m.*, spermatangial mother cell); (All ×900).

THE DEVELOPMENT OF THE CARPOSPOROPHYTE

At the present stage of the investigation it is not possible to state that the further development of the carpogonium is dependent upon fertilization, but the frequent occurrence of trichogynes with fused spermatia suggests that fertilization does take place.

The carpogonium is overgrown very quickly by the continued growth of the lateral branches of limited growth; during this process the trichogyne is broken off at the constriction between it and the carpogonium (Figs. 2*a* and *b*). The carpogonium enlarges rapidly and becomes multinucleate. With this increase in size of the carpogonium, there is evidence that the swollen carpogonium may fuse with the hypogynous cell or other neighbouring vegetative cells (Figs. 2*c*, 3*b*, and *e*), although in other cases it is obvious that such fusions have not occurred (Figs. 2*d* and *f*). A large, multinucleate, irregularly-shaped cell is formed, which may be up to 30μ in diameter. At this stage the outline of the carpogonium is still recognizable occasionally (Fig. 2*e*), although in most examples, the multinucleate cell bears little resemblance to the carpogonium from which it has originated (Figs. 3*a*, *b*, and *e*). Long non-septate processes develop from all parts of the multinucleate cell and these ramify on both sides of the axial plate (Figs. 2*e*, *f*, 3*a*–*e*).

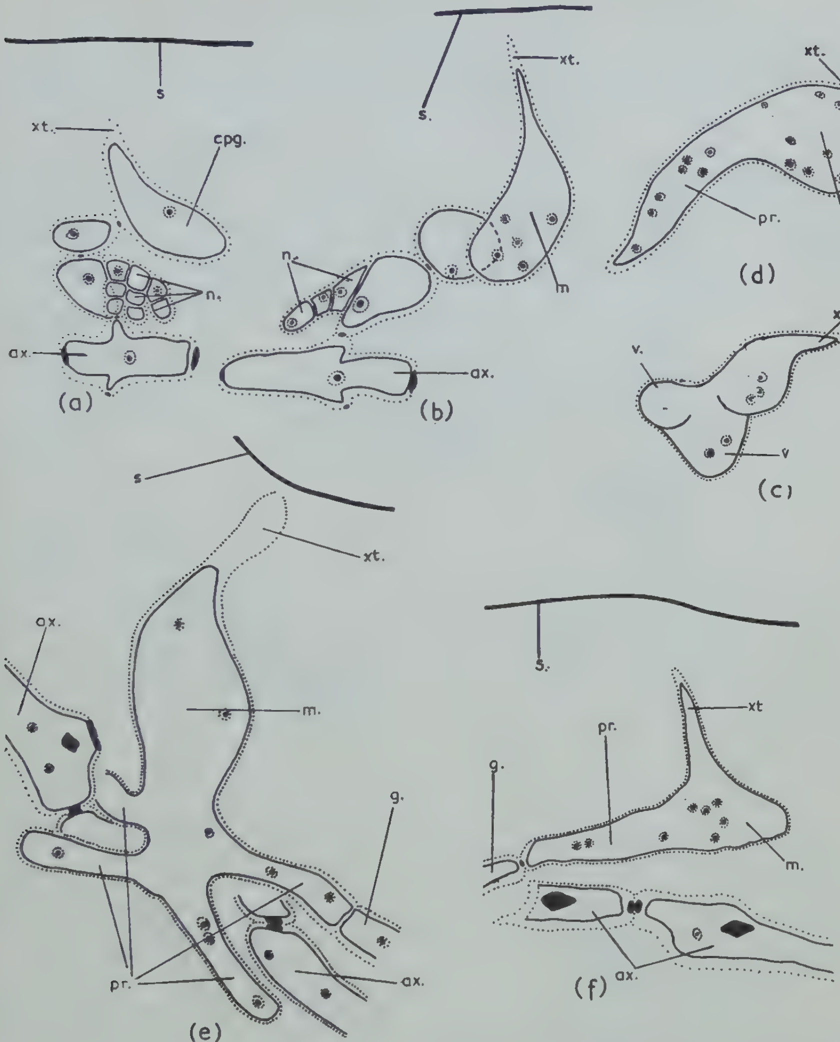

FIG. 2. Early stages in the development of the carposporophyte: (*a*), (*b*), overgrown uninucleate and multinucleate carpogonia; (*c*) multinucleate cell formed by the fusion of the carpogonium and two neighbouring vegetative cells; (*d*), (*e*), (*f*), development of processes from the multinucleate cell: (*a, b, e, f, G. pulchellum; c, d, G. latifolium* (squash preparations)); (*ax.*, axial cell; *cpg.*, carpogonium; *g.*, gonimoblast; *m.*, multinucleate cell; *n.*, nutritive tissue; *pr.*, process of the multinucleate cell; *s.*, surface of the thallus; *v.*, vegetative cell, fused with the multinucleate carpogonium; *xt.*, remains of the base of the trichogyne); (All × 900).

Nuclear divisions take place rapidly, so that a large number of nuclei may occur in the cell and its processes. The staining reactions of both the multinucleate cell and its processes are very weak. Because of this, the difficulties involved in following the ramifications of the elaborately branched processes through several sections necessitated the use of squash preparations for this part of the investigation. Small primordia, which contain usually a single nucleus, are formed at the tips of the processes (Figs. 3*a*, *c*, and *d*). These primordia function as apical cells, each dividing to form a filament of elongate, uninucleate cells which constitutes the gonimoblast (Fig. 3*c*). Lateral branches of the gonimoblast are formed in profusion and a dense mass of interwoven filaments results. The gonimoblast becomes two or three cell-layers deep on each side of the axial plate. During the development of the gonimoblast, the large multinucleate cell and the cells of the nutritive tissue disappear gradually. Clusters of empty, thin-walled nutritive cells occur occasionally, intermixed with cells of the gonimoblast; the nucleus and all but a remnant of the cyto-

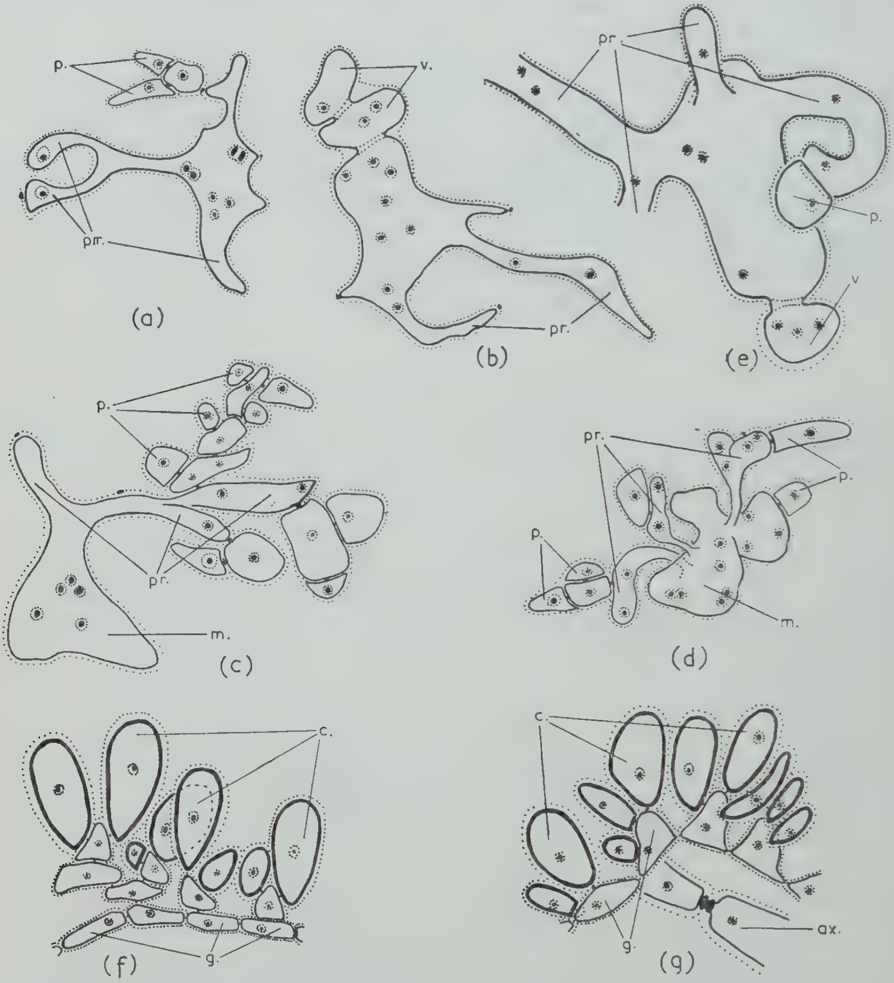

FIG. 3. Later stages in the development of the carposporophyte: (*a*), (*b*), (*c*), (*d*), development of the gonimoblast from the multinucleate cell, by the formation of primordia and their segmentation; (*f*), (*g*), gonimoblast and carpospores: (*a, b, c, d, g, G. latifolium; e, f, G. pulchellum*; (*a–e*, squash preparations)); *ax.*, axial cell; *c.*, carpospore; *g.*, gonimoblast; *m.*, multinucleate cell; *v.*, vegetative cell, fused with the multinucleate cell); (All ×900).

plasm have disappeared. No evidence of direct fusions or connexions between the nutritive tissue and the developing gonimoblast has been obtained.

The elongate cells of the outer layers of the gonimoblast increase in size, becoming conical in shape, whilst the nuclei divide several times. From three to five uninucleate primordia are formed successively on each gonimoblast cell; these are the carposporangium initials (Figs. 3*f* and *g*). The carposporangium initials are from 4·0μ to 6·0μ in length and about 3·5μ in breadth when first formed, but they enlarge slowly to form the carposporangia. A reticulate plastid develops in each and the contents become dense, due to the accumulation of reserve food materials. The mature carposporangia are very large, from 50·0μ to 70·0μ in length and 20·0μ in breadth.

During the maturation of the gonimoblast, changes take place in the cortical tissues on each side of the axial plate. The lateral branches of limited growth, which are from four to eight cells in length when the carpogonia are formed, continue to elongate during the development of the carposporophyte, so that eventually they reach a length of from twelve to sixteen cells. During the later stages of the development of the carposporophyte, mucilaginous areas develop in the cortex on each side of the axial plate immediately to the outside of the developing gonimoblast and carpospores. The mucilage is of deep yellow colour and its development is much more pronounced in *G. pulchellum* than in *G. latifolium*. Because of the presence of this mucilage, the investigation of the development of the carposporophyte is much more difficult in *G. pulchellum* than in *G. latifolium*. The lowermost cells of the lateral branches of limited growth elongate during the formation of the mucilage, becoming very thin and often disintegrating, so that the outer parts of the cortex break away from the axial plate to form the characteristic bilocular 'cystocarp'. A superficial depression is often present in the wall of the 'cystocarp' in both species, but a definite aperture could not be detected. In both species, entire sporangia are detached from the gonimoblast and accumulate in the cavities of the 'cystocarp'. In old decaying specimens, collected in December, the cavities were full of what appeared at first to be carpospores, but the presence of the pit-connexion showed that the sporangium wall was still intact. It is presumed that in the material investigated, the carpospores are liberated by the ultimate decay of the wall of the 'cystocarp'. Both Kylin (1928) and Smith (1955) figure a definite pore in the wall of the 'cystocarp' of *G. cartilagineum*, and it is claimed that the carpospores are shed through this aperture.

It has been stated, without any direct evidence, that the 'cystocarps' of *Gelidium* and *Pterocladia* are compound structures (Fritsch, 1945, p. 630; Smith, 1938, p. 327, 1955, p. 321). In both *G. pulchellum* and *G. latifolium*, from 10 to 30 carpogonia may be formed in each fertile axis, but it is extremely difficult to determine how many of these will develop and give rise to carposporophytes. In *G. pulchellum*, only one multinucleate cell could be detected in each fertile axis, so that it would appear that for this species the 'cystocarp' is simple. In *G. latifolium*, on the other hand, the fertile axes contained from one to four multinucleate cells, but when more than one was present, it was impossible to say whether all of the multinucleate cells contributed to the gonimoblast, although this would appear to be very probable. The 'cystocarp' in the two species investigated therefore may contain gonimoblast derived from a single carpogonium, or, under certain circumstances, from a number of carpogonia.

Overgrown carpogonia which have failed to develop are found frequently during the early stages of the development of the carposporophyte. Enlargement in the manner described does not take place and the carpogonia become irregular in shape. The trichogyne is lost and the nucleus and dense contents disappear slowly.

THE TETRASPORANGIA

The tetrasporangia of both *G. latifolium* and *G. pulchellum* occur normally in the flattened inflated tips of the terminal lateral axes, although during the winter occasional tetrasporangia may occur also in the main axes. The oval spathulate tips are similar in structure to the cylindrical axes, except that the cortical tissue is more diffuse so that the spathulate tips are almost transparent. The tetrasporangia develop from small single-celled primordia formed laterally on the lateral branches of limited growth (Fig. 4*a*). The uninucleate tetrasporangium initial is 15μ to 30μ in length and from 6μ to 15μ in breadth and completely colourless when first formed, but it increases in size rapidly and becomes opaque due to the accumulation of reserve food materials. As a result of the deep red colour of the mature tetrasporangia and the transparency of the flattened spathulate tips in which they occur, it is possible to identify tetrasporic plants with the naked eye. The tetrasporangium initials are formed almost simultaneously during June and July, but the processes of maturation and dehiscence are slow, so that tetraspores are shed continuously until December, or even into the succeeding year. The initials enlarge at random and divide as the reserve food materials and pigment accumulate. The arrangement of the tetraspores is cruciate in most examples (Fig. 4*b*), but the tetrahedral arrangement occurs also, particularly in *G. latifolium*. Occasionally

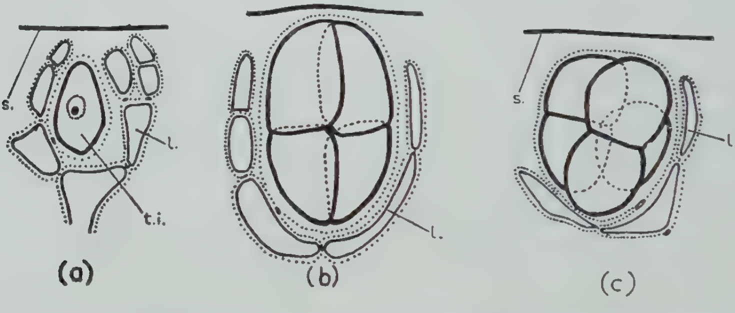

FIG. 4. Development of tetrasporangia in *G. latifolium*: (*a*), formation of tetrasporangium initial; (*b*), cruciate tetrasporangium; (*c*), irregularly divided 'tetrasporangium'; (*l.*, lateral branch of limited growth; *s.*, surface of the thallus; *t.i.*, tetrasporangium initial); (All × 900).

the further division of one or more of the spores gives rise to 'tetrasporangia' containing from five to eight spores (Fig. 4*c*). The tetraspores are liberated by the breakdown of the sporangium wall. Immediately, adjacent initials enlarge to fill the cavity left by the dehisced tetrasporangium. This process continues until all the tetrasporangia have matured and dehisced, by which time the spathulate tips have begun to decay. The diffuse nature of the cortical tissue and the disruption caused by the liberation of tetrasporangia makes the spathulate tips particularly susceptible to decay.

DISCUSSION

The preceding account of the structure and development of the sexual organs and carposporophyte of *Gelidium pulchellum* and *G. latifolium* differs in several respects from the description of the development of *G. cartilagineum* published by Kylin (1928). The principal feature of interest concerns the development of the carposporophyte.

According to Kylin (1928), the gonimoblast of *G. cartilagineum* develops directly from the unchanged carpogonium, as in *Batrachospermum*, but the account given is somewhat fragmentary. The descriptions and figures of the origin of the gonimoblast from the carpogonium are particularly scanty. The only figure given by Kylin of a supposed carpogonium and gonimoblast lobe shows a carpogonium, from which the trichogyne has been lost, with a small prolongation at its base. No nucleus is figured in this carpogonium (Kylin, 1928, Fig. 12D). A figure published by Smith (1938, Fig. 178A; 1955, Fig. 182A) shows a carpogonium in pit-connexion with two gonimoblast cells. No evidence has been obtained, either in *G. latifolium* or in *G. pulchellum*, of the direct development of the gonimoblast from the unchanged carpogonium, as described by Kylin and Smith. Instead, a multinucleate cell of irregular shape is formed, either from the carpogonium alone, or in certain cases by the fusion of the enlarged carpogonium and neighbouring vegetative cells. These fusions are not obligatory and the cells with which the carpogonium may fuse do not appear to be specifically determined. The gonimoblast develops as a result of the activity of apical cells cut off from the tips of the elongate processes of this multinucleate cell.

The structure supposed by Kylin to represent a carpogonium and developing gonimoblast lobe bears a strong resemblance to the overgrown, decaying carpogonia observed in both *G. pulchellum* and *G. latifolium*. These structures are often of very irregular outline as a result of the pressures exerted upon them by the continued growth of the neighbouring lateral branches of limited growth, and are usually devoid of nuclei.

As a result of his investigation of *G. cartilagineum*, Kylin concluded, that in his sense of the term, an auxiliary cell did not occur in *Gelidium*. The term 'auxiliary cell' has been applied by different authors with very different meanings, so that it is necessary to reconsider this conclusion, not only in relation to the new information presented in this paper, but also in relation to the different concepts of the term.

The term 'auxiliary cell' was used first by Schmitz (1883, p. 229), in his description of the development of the carposporophyte of *Naccaria*, to describe the hypogynous cells which fused with the carpogonium. It is clear from his description that Schmitz considered that the function of these cells was principally nutritive. In the majority of Florideae, the auxiliary cell serves also as the starting-point for the development of the gonimoblast. Later authors have used the occurrence and position of the auxiliary cell as the basis for the classification of the Florideae. Kylin (1923, p. 132; 1928, p. 105; 1930, p. 91) redefined the term and restricted its use to those cells which served for the origin of the gonimoblast. These cells were called 'typical' auxiliary cells by Kylin; those cells which were merely storage or nutritive cells were called by the latter name ('Nahrzellen'). The conclusion by Kylin that an auxiliary cell did not occur in *Gelidium* was made on the basis of this definition. Later, when *Asparagopsis* (Svedelius, 1933) proved to have a typical auxiliary cell according to this first definition, Kylin (1935; 1937) added a further requirement: the auxiliary cell could not be a cell of the carpogonial branch. This second definition has been criticized strongly by Martin (1939, p. 139) and Svedelius (1942, p. 85), the latter stating: 'I find it difficult to understand this further restriction of the conception of the auxiliary cell, if it is not to be interpreted as an attempt to retain for the Nemalionales the absence of auxiliaries as a distinct systematic character.' Wilke and Ziegenspeck (1929) and Svedelius (1942) take an equally extreme view, in that they would consider any cell of the female plant which fuses directly or indirectly with the carpogonium as an auxiliary cell. It is not clear how these authors would interpret the vegetative cells of *Gelidium*, which fuse occasionally with the carpogonium, but it would appear from their definitions that these would rank as auxiliary cells. The organization and interrelationships of the carposporophytes of the Florideae have been analysed recently by Drew (1954) who takes an intermediate position, with regard to the nutritive and carposporophyte-forming functions of auxiliary cells, between the extreme views of Kylin and Svedelius. Drew (1954, p. 65) proposes the following definition: 'An auxiliary cell is a specified cell of the gametophyte, with which the carpogonium fuses before the formation of gonimoblasts, or a cell with which the primary gonimoblast fuses. Auxiliary cells have a purely nutritive function in those cases where no nucleus is transferred, or combine nutritive and generative functions in those cases where the fertilization nucleus or its derivative is transferred to that cell and there initiates the development of secondary gonimoblasts.' On the basis of this definition, an auxiliary cell does not occur in *Gelidium*, because the fusions are not obligatory, and the cells with which the carpogonium may fuse are not specifically determined.

The fusions of the carpogonium with the hypogynous cell, or other vegetative cells during the development of the carposporophyte of *Gelidium* are of doubtful significance. They may represent an early stage in the evolutionary differentiation of the auxiliary cell, in that fusions of the carpogonium or gonimoblast with unspecified cells may have preceded fusions with specified cells. It is more likely that they represent nothing more than the accidental absorption of neighbouring cells by the rapid enlargement of the carpogonium.

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Auxin Relations of the Woody Shoot

The Distribution of Diffusible Auxin in Shoots of Apple and Plum Rootstock Varieties

BY

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With four Figures in the Text

ABSTRACT

Surveys have been made of diffusible auxin in the stem tissues of growing shoots of apple and plum rootstock varieties. Using agar plates as carriers auxin was collected from the lower surface of isolated stem sections and assayed by the *Avena* curvature method. The stool and layer shoots studied grew for several months producing many leaves and reaching considerable lengths. The data provide information on selected internodes and show the auxin status of the shoot at various times during growth, and the auxin gradients down the stem at these various times. Free auxin content of the shoot apex was consistently less than that of the internodes below. In 1946 auxin content declined throughout growth with a steady positioning of the auxin peak in the upper shoot. In 1947, following a period of drought, when growth almost ceased, a secondary auxin peak occurred positioned in lower internodes distant from the apex. This seasonal contrast was reflected in the auxin relations of the individual internode, and was observed both in apple and plum. The nature of the auxin decline below the peak region, and the total disappearance of free auxin from the shoot as growth subsides, is discussed. The reappearance of free auxin in mature internodes, which has not been transmitted from the stem apex, implies either derivation from a stored state or the ability of the internode to produce its own auxin.

INTRODUCTION

WHEN this study began in 1946 it was already well known that diffusible auxin is present in the growing shoots of trees, especially in the young buds in spring after the winter rest (Czaja, 1934; Zimmermann, 1936; Söding, 1937). Characteristically a rapid increase in auxin to a peak value occurs about the time of 'bud-burst' followed by a decline. This transient production of auxin is distinct from the auxin relations during the subsequent phase of elongation. The question of auxin distribution in the elongating shoot at once arises, and both Zimmermann and Söding noted that the amount of diffusible auxin obtainable from the lower region could exceed that from the apex. Of particular interest is a study of diffusible auxin in shoots of apple and chestnut (Avery *et al.*, 1937); the main emphasis in this work was upon the bursting bud as the centre of production of auxin passing to the older thickening wood, with less emphasis on the variations of auxin distribution in the newly formed elongating shoots. This established a gradient from the

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actively growing shoot into the wood of the previous year and regarded the auxin thus supplied as a major factor in cambial stimulation. Working with buds of pear and cherry Bennett and Skoog (1938) studied in more detail the winter rest period and showed that the appearance of auxin in the buds could be prevented by withholding normal 'chilling' requirements.

The pattern of auxin distribution in the young woody shoot during development began to engage increasing attention. In his study of two pine species, Mirov (1941) showed that the maximum of diffusible auxin was not located in the tip itself, but occurred lower in the new shoot, and diminished downwards into the previous year's growth. These 'long' shoots were determinate in their growth consisting wholly of the extension of internodes already present in the bud; the 'short' or dwarf shoots bore the axillary needle clusters and these gave low auxin values. Like the buds, the stem tissues were found to contain free diffusible auxin only during growth and not in the resting condition (Oserkowsky, 1942, working with twigs of apple and pear) and vigorous growth was correlated with high auxin yields (Shalucha, 1946, in a study of peach shoots). Both Oserkowsky and Shalucha were primarily concerned with specific questions, the former on the movement of auxin in the stem, the latter on the possible relation of auxin with nitrogen status; whereas Gunckel and Thimann's study (1949) of auxin production in shoot growth of *Ginkgo* was aimed directly at the elucidation of the role of auxin in controlling growth, in particular as affecting relations between 'long' and 'short' shoots.

Essentially, the 'short' shoot of Ginkgo may be regarded as the first phase of development, only certain shoots entering the second or elongation phase to become 'long'. As the buds develop their auxin content increases to a maximum in the 'green' condition, declining again as the bud opens. At this stage the 'long' shoot yields rather more auxin than the 'short' shoot, but as elongation proceeds the auxin increases sharply, not in the bud and youngest leaves, but in the lower internodes, with a peak just above the base where elongation is nearly complete. The Ginkgo 'long' shoot is another example of elongation from a telescoped condition and Gunckel and Thimann concluded that the auxin produced during this extension growth must be released in the stem tissues themselves and not supplied by the apex and young leaves. Essentially the same finding held for the Katsura tree of Japan, studied by Titman and Wetmore (1955).

In a final paper on Ginkgo, Gunckel *et al.* (1949) go on to consider its growth habit in relation to the proportion of 'long' and 'short' shoots, the broadening of the tree with age deriving directly from a decreasing number of the former. In a young tree the long shoots predominate; in a mature flowering tree there are about equal numbers of 'long' and 'short' shoots which pruning aims to maintain in the required balance. The characteristic of tree form is, therefore, determined by the proportion of shoots which elongate from the bud, a proportion which is greatest in the young tree and which progressively diminishes. Gunckel *et al.* interpret the 'short' shoot as a condition of delayed inhibition imposed by the 'long' shoot, as distinct from the condition of total inhibition in the lateral bud. Such full inhibition of the lateral bud is associated with vigorous terminal (apical dominance), removal of the apex being most effective in inducing lateral growth when the tree is young and *vigorous*.

Except as specimen plants, or in special experiments, rootstocks do not acquire tree form, the fruit tree itself being composite of scion on stock. Instead, the source plants from which the newly rooted stocks derive are specially cultured as stools, or layers, or as hedges, from which, in the autumn or winter, all the shoots are harvested. In the next season a new crop of shoots is produced, these shoots being naturally vigorous and essentially vegetative so that, during a growing period of 5 or 6 months, great lengths are attained and many leaves are produced.

It is because the regenerative behaviour of these shoots, in initiating adventitious roots, is conditioned by the action of auxinic substances and can be influenced by treatment with synthetic growth-regulating compounds, that their natural auxin relations required to be examined. At the outset it was soon established that, using an agar plate as carrier, auxin could be collected from the lower surface of an isolated section of stem and assayed by the *Avena* curvature method (Hatcher, 1948). Accordingly surveys of diffusible auxin were made in apple and plum rootstocks which showed the existence of auxin gradients along the shoot and the eventual decline of auxin as the shoot approached winter dormancy.

Materials and Methods

In the main two types of material were used, namely Crab C apple and Myrobalan B plum, the rooting responses of which to synthetic growth-regulating substances were known (Garner and Hatcher, 1955). Cultural origin was in the case of the apple a 'stool' bed, and in the case of the plum a 'layer' bed.

Shoots were removed for auxin estimation at various times throughout the growing season. Growth continued for several months and some shoots reached a length exceeding 150 cm. The portions of shoot sampled were fixed relative to the apex. The 'tip' section included the youngest expanded internode; below this region the next section taken was from the third or fifth internode and then every fifth or tenth internode was sampled. These are referred to as I_3, I_5, I_{10}, &c. The stem sections other than the 'tip' were isolated by severing the appropriate internode at the midpoint and again at 1 cm. above this. All leaves were removed. The material was mounted basipetally on agar plates (1·5 per cent.) of sufficient size to cover the whole cross-section of the stem segment. Collection of auxin was carried out in the laboratory for 1 hour, after which the plates were left for a minimum of 1 hour to allow uniform distribution of auxin to occur, and were then cut into unit blocks, 10 c. mm. in size, for assay.

The method of auxin assay was a modified standard Avena coleoptile curvature test (Went and Thimann, 1937) using seedlings raised in moist sand instead of in water (Hatcher, 1945; Rawes and Hatcher, 1949). The range of curvature of the assay was equivalent to that given by 0·2–0·8 mg./l. (p.p.m.) of indole-3-acetic acid (IAA). The results are presented in terms of Total Degrees Curvature (T.D.C.) per 1 cm. section length per hour.

EXPERIMENTS WITH CRAB C APPLE

Systematic estimations of auxin were carried out with Crab C apple during the seasons 1946 and 1947. Several assays were made during each month from April or May until November, and the mean monthly values of auxin content per 1 cm. stem length are entered as T.D.C. in Tables 1 and 2.

TABLE 1

Diffusible Auxin in Stool Shoots of Crab C Apple in 1946; Average of Assays in Monthly Periods at Various Internodal Positions Downwards from the Tip, expressed as Total Degrees Curvature per 1 cm. Section per Hour

	Tip	I_5	I_{10}	I_{15}	I_{20}
May . .	75·2	82·0	26·4	—	—
June . .	49·2	101·8	48·7	29·8	—
July . .	61·8	73·1	48·2	24·3	21·3
Aug. . .	28·9	33·5	31·5	18·4	12·6
Sept. . .	23·9	33·4	19·3	19·6	10·2
Oct. . .	7·0	13·9	11·7	6·0	2·5
Nov. . .	1·3	2·5	1·9	0·86	0·74

TABLE 2

Diffusible Auxin in Stool Shoots of Crab C Apple in 1947; Average of Assays in Monthly Periods at Various Internodal Positions Downwards from the Tip, expressed as Total Degrees Curvature per 1 cm. Section per Hour

	Tip	I_3	I_5	I_{10}	I_{15}	I_{20}	I_{25}	I_{30}	I_{35}	I_{45}
April	21·0	29·4	—	—	—	—	—	—	—	—
May	58·4	106·6	51·8	20·8	—	—	—	—	—	—
June	51·8	40·5	77·3	70·4	26·2	36·8	27·9	—	—	—
July	51·9	46·4	73·1	64·2	37·5	34·2	10·0	4·0	—	—
Aug.	31·1	—	33·9	42·9	56·4	—	64·1	—	5·6	—
Sept.	22·8	—	42·1	31·5	38·9	—	27·3	—	35·7	47·1
Oct.	0·0	0·0	7·8	6·6	33·4	7·1	12·5	—	8·4	7·5
Nov.	0·0	—	0·0	0·0	0·0	0·0	0·0	—	0·0	0·0

Reading down the columns the changes in diffusible auxin during growth at comparable points along the stem are indicated; while entries along a given row show the gradient of diffusible auxin in the stem at that particular time. It is evident that with time the free auxin content of internodes similarly situated with reference to the apex tends to fall; but in 1946 some internodes, and in 1947 all the internodes show initially a rise in free auxin with a maximum which, in the second year but not the first, is progressively delayed the lower the position of the internode. For throughout the 1946 season auxin values along rows reached a maximum at the fifth internode (I_5) but in 1947 the position of the maximum moved down the stem as the season advanced—in May it occurred in I_3, in June and July in I_5, in August in I_{25}, and in

September in I_{45}. It is noteworthy that the stem tip in every case contained less free auxin than the internodes below.

Interpretation of the values in Tables 1 and 2 is complicated by the fact that each monthly entry for a particular internode below the apex refers to an internode of different age, the successive entries down the columns referring to 'older' internodes in the sense that at the time of their formation in the apex the whole shoot had become progressively older.

From the data collected it was, however, possible to follow the changes in diffusible auxin in separate internodes as they become older. At the time of sampling the total internode number of each shoot was noted and so the position occupied by a given internode in relation to the shoot apex could be determined for any later time. As the sampling occasions were not absolutely regular, mean monthly dates were calculated on an arithmetic basis and expressed on a time scale in days, May 1 being designated day 1. The equivalent monthly means for 'total' internode number were also calculated and, from the curve relating internode number with time, values could be read off corresponding to regular intervals of 4 weeks.

TABLE 3

Internode Production during Growth, Internode Increment in Successive 4-weekly Periods, and 'Serial' Positions of the Apical Internode on Subsequent Occasions for Crab C Apple Shoots in 1946

Occasion wks from 30 April)	Total number of internodes	Internode increment	Subsequent 'serial' positions of the apical internode (I_1) from each occasion of assay.					
4	12		1					
8	19	7	8	1				
12	28	9	17	10	1			
16	39	11	28	21	12	1		
20	46	7	35	28	19	8	1	
24	49	3	38	31	22	11	4	1
28	52	3	41	34	25	14	7	4

To illustrate this, in Table 3 are given for Crab C 1946 data the mean total internode values at intervals of 4 weeks (column 2), together with the mean increment values in successive periods of 4 weeks (column 3), and also the subsequent positions on later occasions of the apical internode (I_1) (columns 4–9). Likewise, the 'serial' positions from occasion to occasion were obtained for all the internode positions assayed, viz. I_1, I_5, I_{10}, &c.

In exactly the same way the mean monthly auxin values for each internode assayed (I_1, I_5, &c.) were plotted against time, and by interpolation values obtained corresponding with the precise occasion interval of 4 weeks. Auxin gradient curves were then constructed relating free-auxin content with internode position for each 'ideal' occasion, and values were read off from these curves associated with the 'serial' positions from occasion to occasion of each of the internode positions assayed. In this way the progressive change

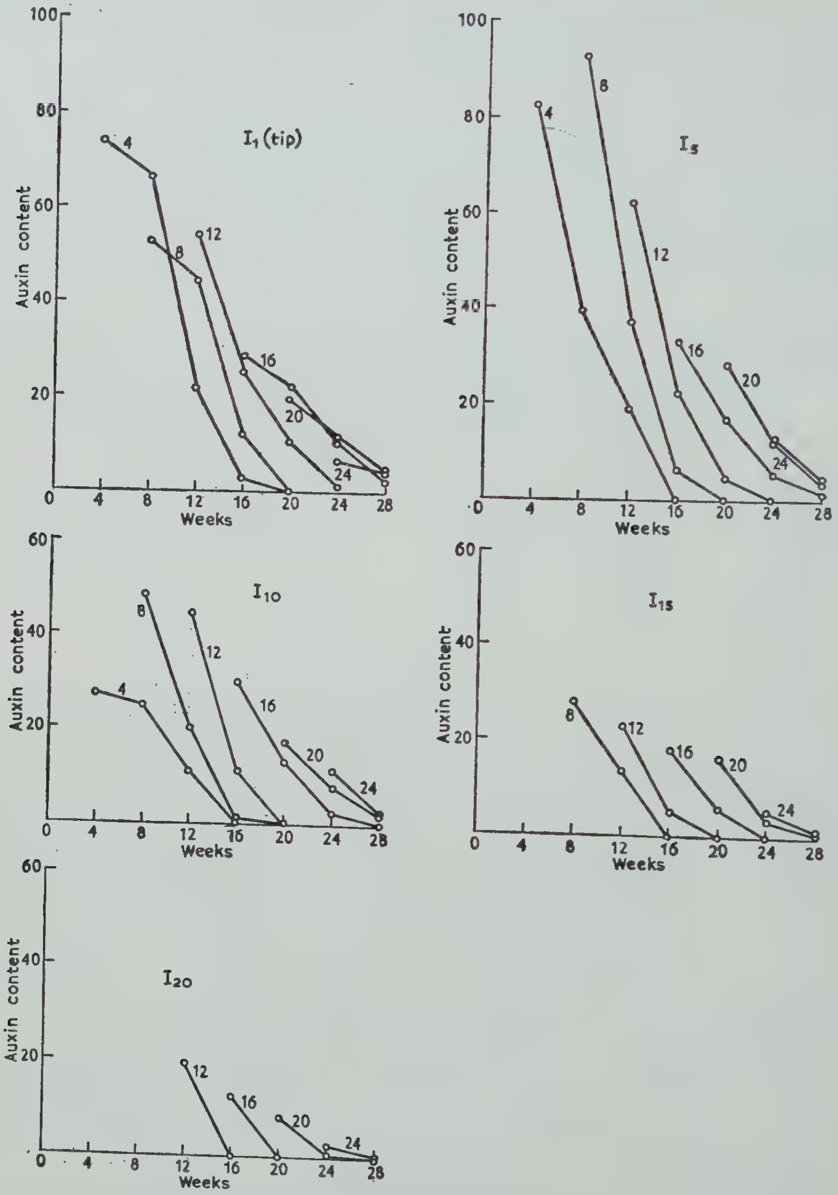

FIG. 1. Progressive change in auxin content (T.D.C. per cm. per hour) of internodes of stool shoots of Crab C apple in 1946; the internodes occupying known positions relative to the apex (I_1, I_5, &c.) from shoots of different age measured in weeks after 30 April when sampled (4, 8, &c.).

in auxin content of internodes occupying known positions relative to the shoot apex was found for shoots of different sizes, the age increment being 4 weeks. These values for I_1 to I_{20} are shown for 1946 in Fig. 1, and for I_1 to I_{35} for 1947 in Fig. 2.

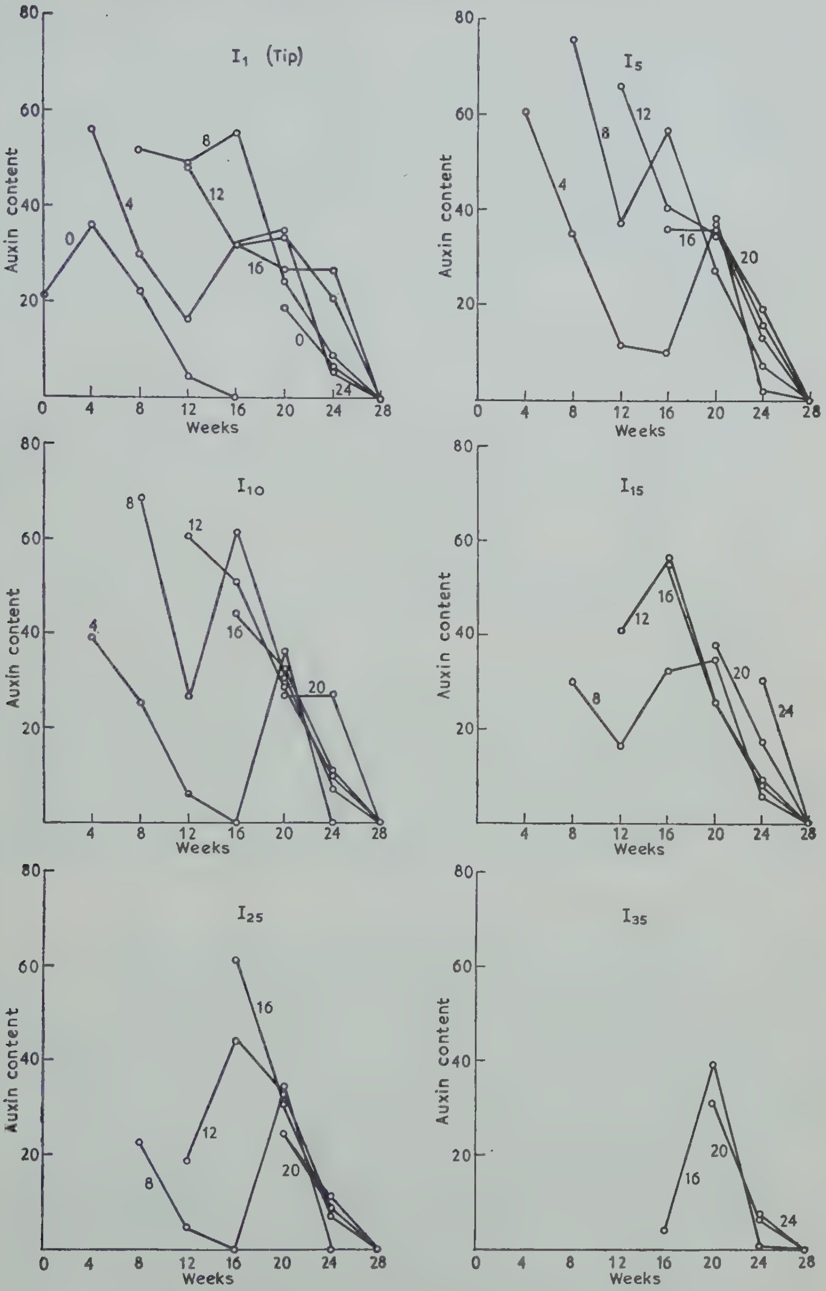

FIG. 2. Progressive change in auxin content (T.D.C. per cm. per hour) of internodes of stool shoots of Crab C apple in 1947; the internodes occupying known positions relative to the apex (I_1, I_5, &c.) from shoots of different age measured in weeks after 30 April when sampled (4, 8, &c.).

The various curves show the auxin content of specified internodes, e.g. I_5, at the time when it initially occupied that position on the stem, and also its subsequent history as it occupied lower and lower positions on the stem. The numerals attached to the separate curves show the age of the shoot (weeks after 30 April) at the time of sampling so that for each internode group (I_5, I_{10}, &c.) the auxin content at 4-weekly intervals is shown, and the change in auxin content of each individual internode as it occupies lower positions on the shoot is given.

Experiment of 1946

Regarding first the experiment of 1946 (Fig. 1) the following conclusions may be drawn:

1. Throughout the whole period of growth of the shoot the internodes similarly situated with respect to the apex display a constant pattern of diffusible auxin content, the maximum always occurring at a short distance (I_5) below the apex. This is unequivocally shown by the entries in Table 1.
2. As growth of the shoot proceeds the youngest internode shows a progressive decline in auxin content, and the lower internodes show a similar behaviour except that the auxin content at first rises, reaching a maximum in June.
3. When the auxin content of each individual internode is followed, it is seen that throughout the whole stem a steady decline in auxin occurs, and the rate of decline as shown by the slopes of the curves is for a long period nearly constant and only falls towards the end of the season.
4. In internodes I_5, I_{10}, and I_{15}, the maximum auxin content occurs in June, the auxin peak thus coinciding with the period of longest day-length.

Experiment of 1947

The data for the experiment with Crab C apple in 1947 are similarly presented in Fig. 2 and Table 2. Whereas in 1946 the shoots were already 20 cm. long with some twelve internodes when first sampled in May, in 1947 the sampling began in April when the shoots had only three internodes.

Changes in diffusible auxin are somewhat more complex though again there is less auxin in the shoot apex than in the internodes below.

In the 1947 experiment two maxima of diffusible auxin occur, one not lower than I_5 during May to July, while later in the season a second maximum occurs which is progressively lower in the stem as time advances.

The curves in Fig. 2 clearly show the secondary rise in auxin content, the first auxin minimum occurring around the 12th–16th week, i.e. in July and August. The steady decline in auxin observed in 1946 is thus interrupted in 1947 by a secondary peak; but thereafter, as in 1946, a rapid decline of auxin occurs in all internodes throughout the whole stem, reaching zero by early November.

Seasonal effect on auxin content of apple

The differences seen in the 2 years may be related to the climatic conditions obtaining during the growth period. In Fig. 3 the rate of internode production is shown and it will be noted that in 1947 up to July the rate of growth was much higher than in 1946, and that after this growth was very slow during August and recovered in September. The final internode number in 1947 was 65 compared with 52 in 1946.

During 1946 the temperature was relatively low, rain fell intermittently throughout the summer months, with 5 inches (twice the normal average) in August, and the only period of sustained sunshine was in July.

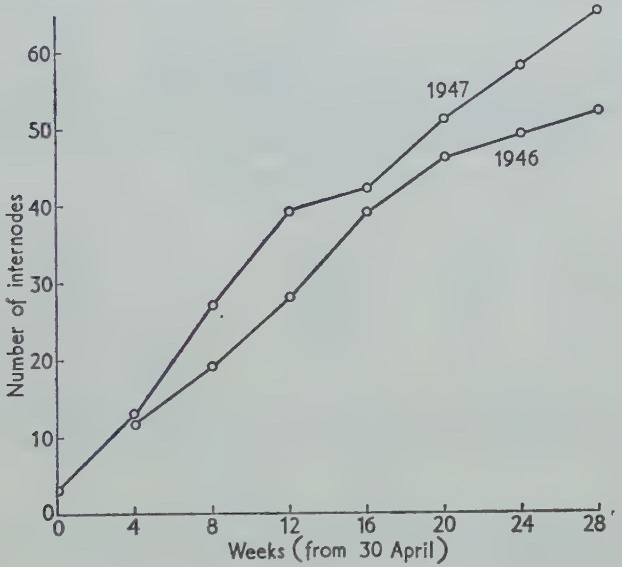

FIG. 3. Comparative growth, measured as internode number, of stool shoots of Crab C in 1946 and 1947; while generally the shoots grew faster in 1947 there was a marked interruption of growth caused by August drought (weeks 12–16).

In 1947 much of the summer rain fell in a few heavy thunderstorms, and there were periods of drought, a completely rainless August giving a record total of 281 hours of bright sunshine. The drought period led to cessation of growth (Fig. 3) and covered the period of the first auxin minimum (Fig. 2). With resumption of rapid growth the secondary auxin peak appeared.

The chief difference between the 2 years is thus the delayed decline of free auxin in the lower regions of the stem in 1947. On the other hand, no marked differences are seen in the auxin content of the upper internodes in the 2 years.

EXPERIMENTS WITH MYROBALAN B PLUM

In 1947 a similar survey was made of layer shoots of Myrobalan B plum. Assays were continued from April to October, during which time the shoots had reached lengths of 150 cm. with nearly 100 internodes, nearly twice as many as in the apple Crab C. Nevertheless, growth had almost ceased by the end of August.

The data for free-auxin content of the internodes as monthly means are entered in Table 4.

Three main observations may be made about diffusible auxin in the plum:

1. The free auxin content of the stem apex is less than of the internodes below, as in the apple.
2. The maximum auxin content in the upper internodes (above I_{15}) occurs around June and July, while in the lower regions of the stem the maximum is found in August in spite of the fact that growth by this time has almost ceased. Again there is some resemblance with Crab C apple in the same year.

TABLE 4

Diffusible Auxin in Layer Shoots of Myrobalan B Plum in 1947; Average of Assays in Monthly Periods at Various Internodal Positions Downwards from the Tip, expressed as Total Degrees Curvature per 1 cm. Stem Section per Hour

	Tip	I_3	I_5	I_{10}	I_{15}	I_{25}	I_{30}	I_{40}	I_{50}	I_{75}
April	22·5	38·0	—	—	—	—	—	—	—	—
May	32·3	28·6	23·1	21·2	24·4	—	—	—	—	—
June	16·7	11·2	27·9	32·0	34·6	54·1	54·2	43·4	—	—
July	41·5	28·0	60·4	—	56·8	—	65·6	29·5	11·0	7·0
Aug.	37·8	—	27·0	—	47·1	—	43·9	—	37·4	41·1
Sept.	0·0	—	0·0	—	0·0	—	3·4	—	16·4	22·1
Oct.	0·0	—	0·0	—	—	—	0·0	—	—	0·0

3. The general level of diffusible auxin is lower in plum than in apple in the tip and upper internodes, but further down the stem the auxin levels are comparable.

The change in diffusible auxin content in individual internodes of the plum are shown in Fig. 4, these diagrams being constructed in the same way as was described for apple.

Resemblance of the curves in Figs. 2 and 4 is close, the plum also showing a double maximum of auxin, with a minimum about the 12th week corresponding with the summer drought period. The subsequent rise in auxin content in the lower portions of the stem was not in the plum, however, accompanied by an effective resumption of growth.

The broad similarity between the data for apple and plum in 1947 lends strong support to the view that the differences seen in the Crab C data in the 2 years was a seasonal effect related to the climatic conditions. This conclusion is further supported by observations on some plum shoots in 1946, the shoots assayed being laterals on 1-year-old nursery plants established from hardwood cuttings. These laterals made only limited growth of up to 25 cm. with 18–20 internodes. The auxin data are presented in Table V.

Although not strictly comparable with the Crab C shoots grown in 1946,

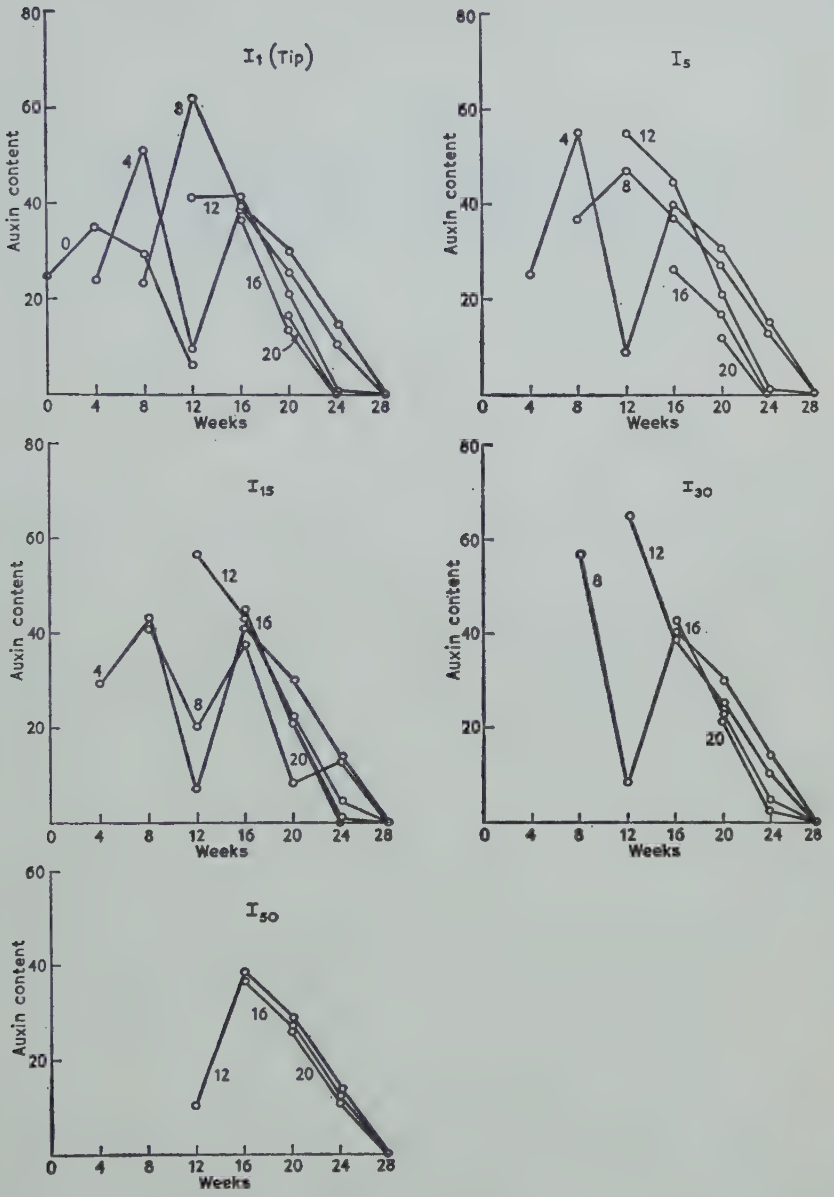

FIG. 4. Progressive change in auxin content (T.D.C. per cm. per hour) of internodes of layer shoots of Myrobalan B plum in 1947; the internodes occupying known positions relative to the apex (I_1, I_5, &c.) from shoots of different age measured in weeks after 30 April when sampled (4, 8, &c.).

the pattern of diffusible auxin in the two cases is very similar. The decline in auxin is continuous in all internodes and no sign of a secondary maximum in the lower internodes is apparent.

TABLE 5

Diffusible Auxin in Lateral Shoots on Cuttings of Myrobalan B Plum in 1946; Average of Assays in Monthly Periods at Various Internodal Positions down the Shoot, expressed as Total Degrees Curvature per 1 cm. Stem Section per Hour

	I_3	I_8	I_{14}	I_{18}
May . .	39·2	36·8	13·6	—
June . .	6·3	7·5	5·7	3·3
July . .	1·6	3·2	—	1·1
Aug. . .	0·0	1·7	—	0·0

DISCUSSION

Attention in this work has been devoted to diffusible auxin in the different internodes during the growth of stool shoots of Crab C apple and layer shoots of Myrobalan B plum. In the assay method employed, namely a modified *Avena* curvature test, the auxin was collected from 1 cm. segments of the stem for a single period of 1 hour. It is known that this is not an exhaustive diffusion but the values obtained (total degrees curvature per cm. length per hour) are comparable and show the relative free-auxin contents of the tissues concerned. The data provide information on internodes in serial order downwards from the tip at different times in the growing season (columns in Tables 1, 2, and 4), as well as on the gradients of auxin down the stem at these various times (rows in Tables 1, 2, and 4). It has been possible also to establish the auxin changes in particular internodes throughout their subsequent history to final maturity (Figs. 1, 2, and 4). The group of curves relating to a given internode represent the auxin contents in that internode from the time at which it occupied the stated position below the apex, onwards. To each individual curve is attached a figure which shows the occasion (weeks from 30 April) when the internode was first assayed, at which time its position on the stem was that indicated by the internode number.

Comparing the two rootstock varieties, auxin values in the upper region of the shoot are always higher in the apple than in the plum, whereas lower down the shoot the values are comparable. In the apple a pronounced seasonal effect was observed, for in 1946 the decline in auxin content continued throughout the growing period, whereas in 1947 a secondary peak occurred at the end of August. In the full experiment with the plum in 1947 a similar late peak of auxin was observed and its seasonal nature also seemed probable from the absence of a secondary peak in 1946 in assay data on lateral shoots of established Myrobalan B cuttings (Table 5). The auxin effect observed in 1947 was associated with a total drought during August which coincided with minimal auxin values and also with the cessation of growth (Fig. 3). With the onset of rain, growth in the apple was resumed and auxin content at the same time increased. It is, however, noteworthy that the secondary auxin peak both in apple and plum occurred in internodes relatively distant from the apex, and not in the proximal internodes, the site of any subsequent growth.

Of great importance is the information obtained on changes in diffusible auxin content in the individual internode during its history. In 1946, over a period of 4 months, auxin content fell at a steady rate and this rate of decline was independent of when the internode occupied the given fixed position (in terms of internode serial number) below the apex, i.e. all curves within the group tend to be parallel (Fig. 1). After August, when auxin content had already fallen to low values, the rate of decline also diminished. In 1947, the picture is complicated by the occurrence of the secondary auxin peak already discussed, though here too there was ultimate total decline.

It is generally agreed that the seat of auxin production is in the stem apex and young developing leaves. The peak of diffusible auxin content, however, is not found in the apex but several internodes below in the region at which the leaves have attained their full size. While this peak is supposedly due to accumulated auxin delivered from the growing zone, the cause of the declining auxin gradient lower in the stem is not clear, and might be attributable to one of three factors. First, auxin may be utilized during the process of growth, or may be destroyed by the so-called auxin oxidase; second, the capacity for transmitting auxin may be lost as the internodes age; and third, the auxin may be converted from the free diffusible form to a bound condition.

As the work of Gregory and Hancock (1955) showed, the stem tissues taken from the region of the auxin peak in the upper shoot transmits the native auxin readily, but unfortunately the capacity for lower and older internodes to transmit auxin was not fully studied. Were their capacity for transmission unimpaired, however, a paradoxical position would seemingly arise, for the auxin so transmitted does not accumulate in the lower stem. Yet attempts to account for apical dominance on the basis of auxin inhibition have always assumed ready transmission throughout the stem. Of the two plants studied, the apple displays intense apical dominance, not so the plum; nevertheless, both plants display essentially similar gradients of diffusible auxin.

The decline in auxin production with increasing age of internode occurs at the same rate irrespective of the time at which the internode was first formed and this would indicate that age as such is not the determining factor. Neither do such considerations suggest a loss of power of auxin transmission by the tissues with advancing age. Again, after a period of temporary arrest of growth due to drought, both in apple and plum, diffusible auxin production again sets in, not in the apical region as one might have expected but in the lower region of the stem. It is known that a similar situation arises at the time of emergence from winter dormancy (unpublished work) and the temporary arrest of growth may therefore be regarded as incipient dormancy. The origin of this secondary auxin in the lower portion of the stem is itself problematical. It has certainly not been transmitted from the apex and so recourse must be made to the third suggestion, namely that the auxin is converted to a bound form which can again be set free. If then this auxin does not originate from the apex it must either have been derived from stored auxin, or the mature internode must be capable of auxin production. Söding long ago suggested that auxin may arise in the process of cambial activity, but if so it is difficult to understand why diffusible auxin is not found throughout the stem as long as secondary thickening continues. Discounting the suggestion, therefore, some kind of auxin release appears the more probable explanation.

Much work has indeed been carried out on the relations during growth of extractable auxin, which will be discussed in a later paper. Suffice it to say that the information on the above question does not altogether clarify the situation, due partly to the presence of inhibiting substances and particularly to the difference in behaviour in this respect of apple and plum. In the former little or no auxin can be detected after ether extraction, whereas in the latter extractable auxin is always present even throughout the period of winter dormancy. In spite of this contrast, however, in the spring at the time of bud-burst ample diffusible and extractable auxin can be detected in both plants.

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Phototropism and Pigment Production in *Sordaria* in Relation to Quality of Light

BY

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With one Figure in the Text

ABSTRACT

Experiments with *Sordaria fimicola* are described giving evidence that in phototropism of the perithecial necks and in the light-induced production of yellow-orange intracellular pigment, light of wave-lengths below 550 mμ is principally effective. This agrees with earlier findings on the sensitivity of light-stimulated spore discharge in this fungus.

INTRODUCTION

IT has been shown (Ingold and Dring, 1957; Ingold, 1958) that whilst light is not essential for spore discharge in *Sordaria fimicola* it strongly stimulates the process. Further, it is the rays of visible light of shorter wave-length, below 550 mμ, which are effective.

At least two other and very different processes in *Sordaria* are light-sensitive, namely the production of yellow-orange colour in the mycelium and perithecia, and the direction of growth of the perithecial necks. The work reported here was undertaken to see if in these two processes the general response to light of different wave-lengths was similar to that of spore discharge.

The same strain of *Sordaria fimicola* was used as in the work reported in the two earlier papers.

PHOTOTROPISM OF PERITHECIAL NECKS

Cultures were grown on filter-paper-yeast-extract agar brimfull in 2·5×2·5 cm. specimen tubes (Ingold and Dring, 1957) at 20° C. with continuous overhead light from 'daylight' fluorescent tubes giving an intensity of about 1,000 m.c. (lux). Spore discharge commenced 8–10 days after inoculation and continued to be reasonably active for about a week subsequently. Only young and vigorous cultures were used in the experiments.

For studying phototropism a black perspex box with a detachable metal ridge-roof fitted with a drawer-like slide-holder was made (Fig. 1). In this box the culture could be subjected to unilateral light through a sky-light carrying a light-filter, or it could be completely darkened by blacking-out the sky-light. In an experiment a specimen-tube culture was fixed in the box, but first the area from which discharge could occur was restricted to a rectangular one (2·5×0·4 cm.) by inverting over it an aluminium capsule, only slightly bigger than the specimen tube, with a slit of the required size cut in its circular bottom. The culture was fixed so that the exposed rectangular area was at right angles to the length of a specially engraved microscope slide held in position in the slide-holder about 3 cm. above the discharging perithecia. Humidity within the box was maintained at a necessary high level by the presence of exposed water surfaces in small specimen tubes. Experiments were performed in a constant-temperature room at 20° C. The culture was then left in the blacked-out box for several hours until discharged spores had produced a deposit of suitable density (normally 1,000–4,000 spores) on the graduated slide. This was then removed and the deposit recorded by counting under the microscope the number of spores in each 2 mm.-wide transverse strip. From these observations a 'mean position' of the spores on the slide could be calculated. Then, with the appropriate light-filter in position, the culture was subjected to unilateral illumination for a period (17 hrs. in one experiment; 20 hrs. in the other), the source of light being a 'daylight' fluorescent tube. After this time a clean slide was placed in the slide-holder, the

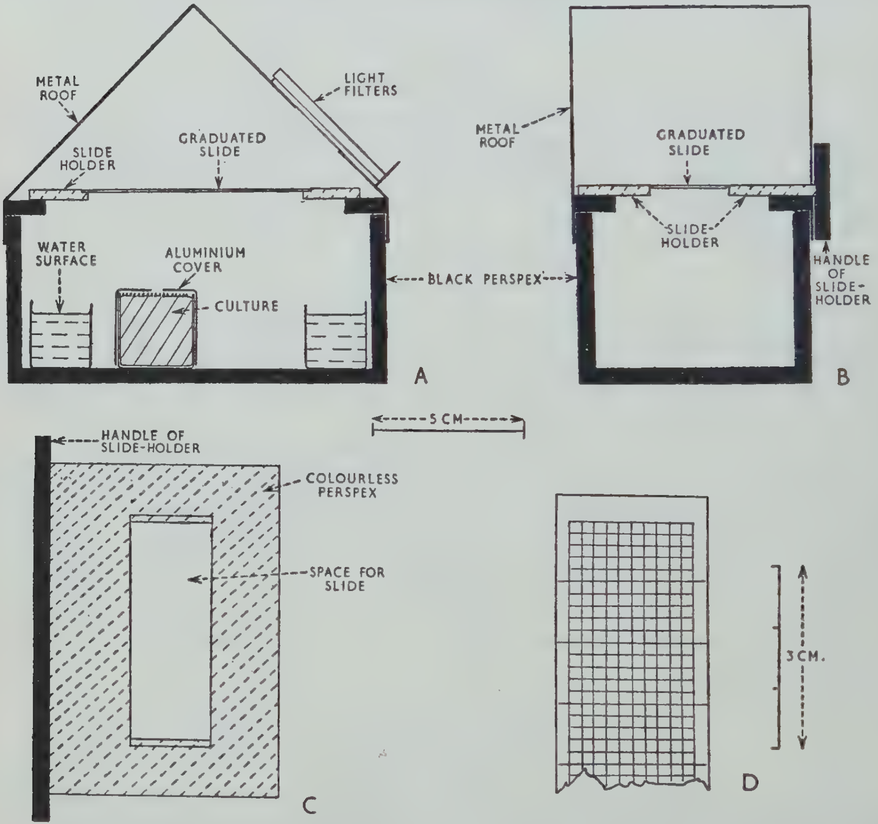

FIG. 1. A, median vertical section of experiment box along longitudinal axis; B, same in plane at right angles (i.e. along transverse axis); C, surface view of slide-holder; D, part of slide, more enlarged than the rest, to show nature of graduations.

box was again blacked-out and transferred to an incubator at 25° C. to encourage rapid spore discharge. An hour or two later the slide was removed and the spore deposit recorded in the manner previously described. A 'mean position' was again calculated, and from this and the earlier observation any shift in this position could be determined. A shift in the mean position of the spore deposit towards the light is due to phototropic curvature of the perithecial necks affecting the direction of ascus discharge.

TABLE 1

Phototropic response of perithecial necks in Sordaria

(Negative values indicate a shift of mean away from light)

Filter	Transmission range	Region of maximum transmission	Shift of mean position of spore deposit in mm.	
			Expt. 1 (20 hrs. illumination)	Expt. 2 (17 hrs. illumination)
Wratten 18 B	240–440 mμ	280–380 mμ	24·3	20·2
Ilford 305	350–510 mμ	440 mμ	26·2	29·4
,, 623	460–540 mμ	495 mμ	22·4	16·4
,, 625	510–590 mμ	540 mμ	−1·8	0·6
,, 626	550–630 mμ	570 mμ	1·9	−2·4
,, 607	570–720 mμ	600 mμ	−0·5	2·6

This procedure was followed for a range of wave-lengths using the colour filters listed in Table 1, a separate culture being used for each test. In all cases a Chance ON20 filter was employed in addition to cut out most of the infra-red rays. In each test with different filters the light intensity was brought to the same value by adjusting distance from the light-source. Since the actual intensities used were low (approximately 30 m.c.) they were measured with a selenium photo-cell connected with a highly sensitive microammeter, instead of using a thermopile. In adjusting the light of different colours to the same intensity, reference was made to the curve for the photo-electric cell relating sensitivity to wave-length.

The results of two experiments are shown in Table 1. It is to be observed that sometimes small negative movements—that is to say, away from the source of light—appear to have occurred, but these slight changes in the mean position of the spore deposit are almost certainly not significant.

For wave-lengths much greater than 600 mμ the selenium cell is not satisfactory in estimating intensity, and no exact determinations of phototropic response were attempted with red filters. However, preliminary tests using intensities of red light considerably above those employed with other colours indicated that the perithecial necks failed to respond to unilateral red light.

In their response to light the perithecia of *Sordaria* agree with other fungi in which phototropism has been studied. For the sporangiophores of *Phycomyces* Castle (1931) found that only light of wave-lengths below 520 mμ was effective with maximum sensitivity in the region of 440 mμ. In *Pilobolus*

Bünning found the same general picture. There was little or no response to light of wave-length above 550 mμ, but at lower values curvature occurred. Using mature sporangiophores (Bünning, 1931), two maxima (at 445 mμ and at 485 mμ) of sensitivity were observed, but in later work (Bünning, 1937) with young sporangiophores the evidence suggested that only a single maximum was involved. In *Coprinus* Borris (1934) found that phototropic curvature of the stipes showed a maximum sensitivity between 440 and 450 mμ, but to light of wave-lengths greater than 590 mμ practically no tropic response occurred.

PIGMENT PRODUCTION

In the study of pigment production the fungus was grown on the usual cellulose-yeast-extract medium with, however, the omission of agar. This medium was dispensed in 25 ml. portions into glass capsules (6·9 cm. diam., 4·0 cm. high) each of which was placed in a cylindrical tin (7·3 cm. diam., 5·8 cm. high) with a loosely fitting lid having a square (4·5 cm. side) cut out of it. After sterilization this hole was covered with the appropriate colour filter (5×5 cm.), surface-sterilized by sponging with ethanol. The filter was held in place by small pieces of 'Sellotape'. Where necessary supplementary 'neutral' filters were used. Each tin was placed in a constant-temperature room (22±1° C.) and illuminated with 'daylight' fluorescent tubes. The intensity was adjusted to be of the same value (approximately 30 m.c.) for the different colours using the same general procedure as in the work on phototropism. In most experiments there was also a 'dark' control. After incubation for 8–11 days the mycelial mat with its attached cellulose fibres was thoroughly washed by a standard procedure. The mat was then sucked or squeezed to eliminate excess water and the pigment was extracted. Two methods were used. In most experiments pigment was removed in boiling ethanol and the extract was then filtered and made up to a standard volume.

TABLE 2

Wave-length of light and orange-yellow pigment production in Sordaria.
Intensity 30 m.c. (lux)

(Cultures in Expts. 1 and 2 were 9 days old when harvested, in Expt. 3, 11 days, and in Expt. 4, 8 days). (In Expts. 1–3 pigment extracted with ethanol; in Expt. 4 with methanol followed by acetone).

Filter	Transmission range	Region of maximum transmission	Pigment production in arbitrary units			
			Expt. 1	Expt. 2	Expt. 3	Expt. 4
Wratten 18B	240–440 mμ	280–380 mμ	14·0	22·2	13·0	16·2
Ilford 305	350–510 mμ	440 mμ	21·0	28·0	21·6	26·2
,, 622	380–530 mμ	465 mμ	—	28·0	18·0	25·2
,, 623	460–540 mμ	495 mμ	14·9	25·0	15·5	17·0
,, 625	510–90 mμ	540 mμ	6·2	15·0	8·0	4·5
,, 607	570–720 mμ	600 mμ	5·5	5·1	4·0	4·2
Dark	—	—	—	5·1	6·0	4·0

In the second method, used only in Experiment 4 (see Table 2), extraction was by methanol followed by acetone at room temperature (Zalokar, 1954). Both methods gave closely comparable results. The amount of pigment in solution was determined in arbitrary units with an EEL photometer using a blue (602) filter.

It is clear from the results shown in Table 2 that as with phototropism of the perithecial necks and also with spore discharge, the production of yellow-orange pigment is affected by light, and it is the shorter visible rays that are effective. It is to be noted that some pigment is produced in the dark and in light of longer wave-lengths (> 550 mμ). There is no evidence that the yellow pigmentation represents a single coloured substance. Indeed, it is very possible that two pigments or pigment complexes are involved, one requiring light for its production and the other not. If this should prove to be so, it may well be that the pigment the production of which is light-insensitive is the photo-receptor in connexion not only with the formation of the other yellow pigment (if there are two types) but also in connexion with the two other light-sensitive processes, spore discharge and neck phototropism. It is hoped to give further consideration to the nature of these pigments.

These observations on pigment production compare well with those of other workers. Zalokar (1955), who gives a detailed action-spectrum of carotenoid production in *Neurospora*, found that the effective region of the spectrum was mainly between 400 and 500 mμ with a broadish maximum in the range 450–90 mμ. From the shape of the action spectrum curve he concluded that the photo-receptor in the process was probably a riboflavin derivative, though not riboflavin itself, and certainly not a carotenoid. Friederischen and Engel (1957) working with *Sphaerobolus stellatus* found that the yellow pigment (probably carotenoid) produced both in the mycelium and also in the fruit-bodies was formed only in light, the effective rays being in the region 365–500 mμ.

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The Net Assimilation Rates of Wild and Cultivated Beets

BY

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With two Figures in the Text

ABSTRACT

The net assimilation rate (*E*) of Kleinwanzleben sugar-beet was the same as that of three types of wild sea-beet (*Beta vulgaris* subsp. *maritima*) when the leaf-area index (*L*) was near to 1. In a subsequent period, when mean *L* of sugar-beet and of the leafiest wild beet type was 2·5, there was an inverse relation between *E* and *L* of the three wild types, and *E* of sugar-beet was then much greater than that of the wild type with equal *L*, but was little different from that of the wild type with smallest *L* (about 1·5). It is concluded that the development of sugar-beet from its wild ancestors by selection and breeding has not affected the intrinsic photosynthetic efficiency of the leaves, but has diminished the effect of mutual-interference between leaves, so that *E* falls less rapidly as *L* increases, i.e. it has decreased the leaf-density dependence of *E*. This change may be related to the difference in form between sugar-beet and wild beet plants.

INTRODUCTION

WHEN Heath and Gregory (1938) collated all the measurements of net assimilation rate (*E*) then available they came to the conclusion that mean *E* during the vegetative phase is nearly the same for all species and environments, and Gregory (1950) therefore assumed that there is little hope of increasing the efficiency of the photosynthetic process by selection or breeding. However, later work has established that there are differences in *E* between species growing in comparable conditions (Watson, 1947; Blackman and Wilson, 1951), between varieties of cultivated species (Watson, 1947) and between progenies of individual plants of a cultivated species (Goodall, 1950), which suggest that some genetic variation in *E* exists on which selection and breeding might operate to improve yield by increasing *E*, and to combine high *E* with other desirable characters. If this is possible, it should have occurred in the course of evolution of cultivated plants, and cultivars might be expected to have higher *E* than wild varieties of the same species.

An opportunity to test this occurred in 1956 when plants of the wild sea-beet (*Beta vulgaris* subsp. *maritima*) were grown at Rothamsted in a field experiment in comparison with cultivated sugar-beet of the Kleinwanzleben-E strain, one of the most productive of those grown in England. The wild beet plants were raised from seed collected in 1955 by Dr. J. W. Blencowe from many different sites near the coast in England and Wales, in connexion with work on beet yellows virus. The sea beet is commonly believed to be the wild plant from which cultivated forms of *Beta vulgaris* have originated (Bell,

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1948) because its seed produces plants of very variable form, some resembling the different types of cultivated beet. However, this similarity could be partly due to fertilization of sea-beet by pollen from cultivated beet, because the wild and cultivated forms readily cross-fertilize.

Material and Methods

Seed from the different collections was sown by hand on one of the Garden Plots at Rothamsted on 17–18 April 1956, in 5-yd. lengths of row, and each collection was replicated a variable number of times up to five, depending on the amount of seed available. The rows were spaced 24 in. apart. Rows sown with Kleinwanzleben-E sugar-beet were interspersed frequently amongst the wild beet. The plot received 10 cwt. per acre of a granular compound fertilizer, containing 7 per cent. N, 7 per cent. P_2O_5, and 10 per cent. K_2O, and 2½ cwt. per acre of agricultural salt before sowing. There was a long dry period immediately after sowing, and few seeds germinated until after rainfall at the end of May. In June there was a large difference in size between the few seedlings that germinated early and those that appeared after the rain. The plants were thinned to 10 in. apart in the rows in late June. Early in July five replicate rows of each of three collections of wild beet were selected for uniformity of size, type, and spacing, and absence of bolting; only plants of the late germination were chosen. The origin of the three selected types was as follows:

1. Seed from a single plant from near Wells-next-the-Sea, Norfolk (*W*).
2. Seed from two similar plants found near King's Lynn, Norfolk (*N*). One plant provided seed for three replicates and the other for two replicates.
3. Seed from three plants collected on the Pembrokeshire coast (*P*). Three replicates were sown with seed from one plant, and each of the other plants provided seed for one replicate.

The seed of types *W* and *N* came from sites near the main area of sugar-beet cultivation in East Anglia, and could have developed from flowers fertilized with sugar-beet pollen or from plants that were hybrids between wild and cultivated beets, but type *P* was from a site remote from sugar-beet or mangold crops and was unlikely to have had cultivated beet in its ancestry.

Five replicate rows of Kleinwanzleben-E sugar-beet (*K*) were also selected. The experiment was arranged in randomized blocks, but the selected types were widely separated within the blocks by plants of other collections, and elimination of block differences did not significantly decrease the error variance of net assimilation rate.

Each replicate of a beet type consisted of fifteen plants divided into three samples of five plants, one of which was harvested on each of three dates: sample I, 26–28 July; sample II, 16–18 August; sample III, 30–31 August. The interval between the first and second harvest was 3 weeks and between the second and third harvests two weeks.

At the first harvest, the total leaf areas of each of the five plants in sample I of a replicate of each beet type were first determined by rating each leaf in comparison with a graded series of leaf shapes of known area (Bald, 1943), and summing the areas of all the leaves. The five plants were then dug up, the roots washed and dried, and each plant was weighed fresh. They were then divided into leaf laminae, petioles and crown, and root; corresponding parts of the five plants were bulked and weighed, and sub-samples were taken to determine the dry-matter percentage of fresh weight for each plant part and hence for the whole plant. The total leaf area of each plant of sample II was also measured by the rating method.

For each of the four types of beet a linear regression of fresh weight per plant on total leaf area per plant was calculated from the weights and areas of the 25 plants in sample I. The fresh weight at harvest 1 of each plant in sample II was then estimated by substituting its leaf area at harvest 1 in the appropriate regression equation. The estimated fresh weights of the five plants in sample II of a replicate were then summed and their total dry weight was calculated from the dry-matter content percentage of fresh weight of the corresponding sample I.

A similar procedure was followed at harvest 2; the total leaf area and fresh weight of each plant in sample II, and the total leaf area of each plant in sample III was measured, and dry-matter content percentage of fresh weight was determined on sub-samples from the bulked five plants of sample II. The total dry weight at harvest 2 of the five plants of sample III for each replicate of every beet type was then estimated in the same way as the initial weights of sample II at harvest 1.

At harvest 3 the plants of sample III were dug up, each was weighed fresh and its leaf area measured, and sub-samples were taken for determination of dry-matter percentage of fresh weight. For convenience, the leaf-area rating on this occasion was done in the laboratory after the plants were dug; at previous harvests it was done in the field, in order that the measurements on harvested plants and on those to be harvested on the next occasion should be strictly comparable.

The mean net assimilation rate (E) in the interval between harvests 1 and 2 was calculated for each replicate of the four types of beet as

$$\frac{(W_2-W_1)(\log_e L_2-\log_e L_1)}{(t_2-t_1)(L_2-L_1)},$$

where L_1 is the leaf area in sq. metres of sample II at harvest 1, W_1 the dry weight in g. of sample II at harvest 1, estimated from the regression equation of fresh weight on leaf area calculated from sample I and the dry-matter percentage of fresh weight of sample I, and W_2 and L_2 are the total dry weight and total leaf area respectively of sample II at harvest 2, and t_2-t_1 is the interval between the harvests in weeks. A similar procedure was used to calculate mean E for the interval between harvests 2 and 3.

Calculation of the dry-matter increment during the experimental interval as the difference between the dry weight of a sample at the end of the interval and its initial dry weight estimated from its leaf area at the beginning of the interval, has been shown to give much more precise values of mean E than if the increment is calculated as the difference between the dry weights of two independent samples, one harvested at the beginning and the other at the end of the interval (McIntyre and Williams, 1949; Watson and Wilson, 1956).

In presenting the results, leaf area per sample has been converted to leaf-area index (L; leaf area per unit area of land), to allow direct comparison with other data from field crops, and E is expressed in g./m.^{2} of leaf/week. To be consistent with these measures of E and L, the dry-weight yield has been given in g./m.^{2} of land.

RESULTS AND DISCUSSION

In the first interval, between harvests 1 and 2, there were no significant differences in E (Table 1) but the mean for sugar-beet was a little less than those of the wild beet types. However, in the second interval between harvests 2 and 3, sugar-beet had the highest mean E, and the difference between it and the mean of all the wild types was significant ($P < 0{\cdot}01$). This was mainly due to the low value for type N; the means for K, W, and P did not differ significantly. The means of E over the whole experimental period, weighted by the length of the intervals (Table 1) were almost identical for W, P, and K, but N had a lower mean.

TABLE 1

Mean Net Assimilation Rate g./m.^{2}/week, Leaf-area Index, Yield of Dry Matter g./m.^{2} at Harvest 3, and Number of Leaves per Plant at Harvest 3, of Sugar-beet and Wild Beet Plants

	Net assimilation rate			Leaf-area index							
	Interval between harvests:			Harvest				Dry weight			
Type of beet	1–2	2–3	Weighted mean	1	2	3	Weighted mean	Tops	Roots	Total	Leaf number
Sugar-beet K	56	45	52	0·7	2·2	2·8	1·85	311	182	493	24
Wild beet											
N	57	25	44	0·6	2·2	2·8	1·83	252	83	335	88
W	60	36	50	0·5	1·8	2·4	1·50	275	84	359	100
P	62	38	52	0·3	1·3	1·7	1·08	213	61	274	99
S.E.	3·8		2·7	0·06	0·33	0·56	0·16	60	18	75	12
Mean difference between sugar-beet and wild beet, $K-\frac{1}{3}(N+W+P)$	−3·4	12·4	2·9	..	..	..	..	..	..	..	..
S.E.	4·5		3·2	..	..	..	..	..	..	..	..

The simplest interpretation of these results would be that the photosynthetic efficiency of the leaves has not been improved during the development of high-yielding cultivars, because some types of wild sea-beet have net assimilation rates as high as that of the most productive sugar-beet. However, this interpretation takes no account of the fact that the beet types differed in leaf-area index, and that E has been shown to depend on L.

Experiments in which L was varied by removal of different fractions of the plant population of field crops showed that E of kale decreased as L increased within the range from 1 to 5 (Watson, 1958), presumably because more intense mutual shading decreased the rate of photosynthesis of the lower leaves. With increase of L of sugar-beet over the same range, E decreased much more slowly for beet than for kale and consequently, although the two crops differed little in E when L was near to 1, E was much greater for sugar-beet than for kale when L was near 5. Inverse correlations between E and L have also been found in comparisons between commercial strains of sugar-beet and between varieties of potato (Watson, 1947); for example, large-topped sugar-beet strains bred primarily for high yield had a lower E than small-topped strains bred for the highest sugar content in the root. Within these species, varieties with higher leaf area tend to have larger but fewer leaves and the lower E of these varieties may be partly the result of internal change in the leaves associated with increased leaf expansion, as well as of external change, presumably in illumination, due to closer crowding of leaves, such as occurs with increase in a population of uniform plants on a field plot.

The wild beet types, like the strains of sugar-beet, showed an inverse relation between E and L in both sampling intervals (Table 1); type N had the highest E and lowest L, while type P had the lowest E and the highest L. The linear regression coefficient of E on mean L during the sampling interval calculated from the means for the three wild beet types for the first interval, -9.0 ± 9.7 g./m.^{2}/week/unit of L, was not significant although the regression line fitted the three points closely (Fig. 1) and accounted for more than 90 per cent. of the variance of E between types, because the variance between types was not much greater than the error variance, but the regression coefficient for the second interval, -13.3 ± 5.9, was significant ($P < 0.05$). When mean E for sugar-beet in the interval between harvests 1 and 2 was plotted against mean L (Fig. 1), it lay very close to the regression line fitted to the data for the wild types, but in the interval between harvests 2 and 3 mean E for sugar-beet was well above the regression line for the wild types, and was significantly greater than mean E for wild type N, which had almost the same mean L as sugar-beet. Thus, sugar-beet and wild beet did not differ in E when L was near to 1, but E for sugar-beet was greater than that of the wild beet when L was about 2·5.

The two regression lines for the wild beet did not coincide; the line for the second interval was below that for the first, suggesting that weather conditions had become less favourable for photosynthesis in the second interval and this accords with the known drift of E in July and August (Watson, 1947). If similar external conditions had persisted throughout both intervals, E for sugar-beet in the second would have been nearly the same as in the first. The difference between sugar-beet and wild beet in the relation of E to L, illustrated in Fig. 1, would then be very like that between sugar-beet and kale described above, with little or no change in E for sugar-beet as L increased from 1 to 3, but a steep fall in E for kale and for wild beet. The plants can be compared in respect of the dependence of E on L by expressing the regression coefficient as a percentage of the value of E when $L = 1$. On this basis, the regression coefficient represents the percentage decrease in E when L increases from 1 to 2; for sugar-beet the decrease was only 6 per cent., for kale 12 to 19 per cent. (Watson, 1958; Table 3) and for wild sea-beet, estimated from the differences between the three types in the second interval, 29±13 per cent. The figure for the wild beet was less accurately determined than the others, and was not significantly different from the values for kale. No estimate for sugar-beet is possible from the present experiment; it cannot be made from the difference in E between the two intervals, because this was probably affected by change in climatic factors.

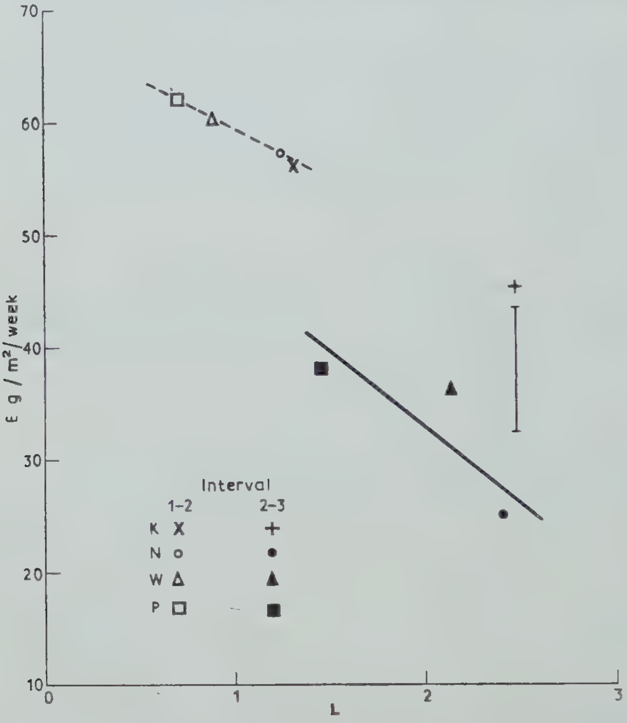

FIG. 1. The relation between net assimilation rate (E), and leaf-area index (L), for sugar-beet (K) and three types of wild sea-beet (N, W, and P). The values of L are means for the period over which E was estimated. The regression lines were fitted to the points for the wild beet types only, for the interval between harvests 1 and 2 (broken line) and between harvests 2 and 3 (full line). The vertical line represents the least significant difference ($P < 0{\cdot}05$) between means of E.

Mutual interference between the leaves of a plant and between neighbouring plants will be minimal when L is less than one, or not much greater than one, and environmental conditions will then be most nearly uniform for all the leaves of a plant and for the leaves of plants of different types. In this state, sugar-beet and wild sea-beet had the same E. Evidently the development of cultivated sugar-beet from wild types has not affected the intrinsic photosynthetic efficiency of the leaves, but has improved the photosynthetic behaviour of the plants in another way; it has diminished the effect of mutual interference between leaves, so that when they are crowded together their mean efficiency in dry-matter production is not much impaired.

If, as seems most likely, leaves mutually interfere by shading each other, the decreased sensitivity of E to increase in L might be due to a change in the relation of the rate of photosynthesis by individual leaves to light intensity, such that sugar-beet has the same rate as wild beet in full daylight, but a higher rate than wild beet in intensities below full daylight. Alternatively, the sensitivity of E to change in L may depend on the form of the plants (cf. Fig. 2) as already suggested for the similar difference between sugar-beet and kale (Watson, 1958). The leaves of a sugar-beet plant, produced from a single apical meristem on an axis that does not elongate, stand side by side in a narrow zone near the ground, the younger ones erect and the older ones progressively more and more inclined towards the horizontal, so that much of the leaf area receives direct sunlight incident at a small angle to the leaf surface. In contrast, the wild beet plants produced leaves from several meristems, and by the last harvest had about four times as many leaves as sugar-beet (Table 1); the leaves were correspondingly smaller in size. The plants had a more prostrate habit, and the leaves tended to lie horizontally, overlapping more than in sugar-beet, so it is probable that a smaller fraction of the leaf area received direct sunlight, though more nearly perpendicular to the leaf surface and therefore at a higher intensity, than in sugar-beet, and a larger fraction received diffuse or only transmitted light. The disposition of leaves of the sugar-beet probably gives a more uniform illumination of the whole leaf area than that of the wild beet. Increase in leaf-area index of sugar-beet will, therefore, tend to distribute the incident radiation over a larger leaf area, but for wild beet it may only add to the layers of shaded leaves illuminated by diffused or transmitted light, with little addition to the area in full daylight. This difference could account for the greater decrease in mean E with increase in L of the wild beet.

A possible criticism of the interpretation of the results is that estimates of leaf-area index derived from single-row plots may not adequately specify the leaf density that determined the degree of shading to which the leaves of the experimental plants were subjected, because this depended also on the leaf area of adjacent rows. However, it seems unlikely that this greatly affected the regressions of E on L, because the spacing between rows was twice that within rows, so that there was much more mutual shading between leaves of the same plant and of neighbouring plants in a row than between rows, and because the plants in the adjacent rows were of similar size to the experimental plants.

It was noted in the introduction that either of the wild beet types W or N, grown from seed collected in Norfolk, might be hybrids derived from crosses with cultivated beet. The only evidence in favour of such a possibility is that the value of E for type W in the second interval lay above the regression line of E on L (Fig. 1), suggesting that this type was intermediate between sugar-beet and the other wild types.

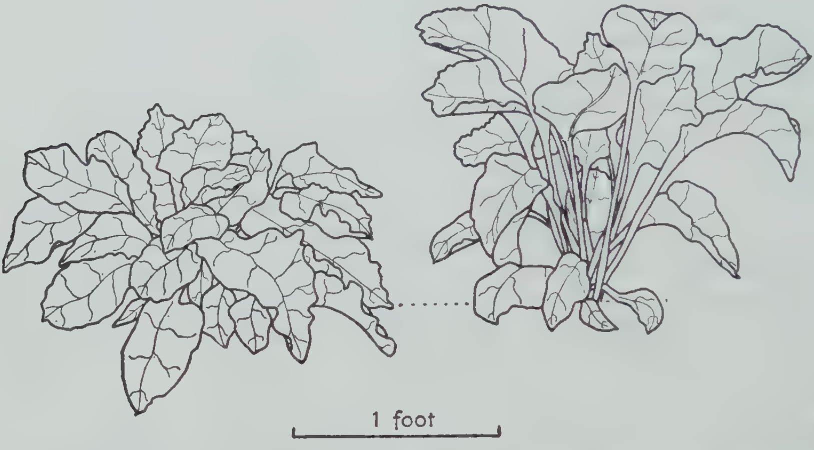

FIG. 2. A tracing from a photograph of the end plants in (left) a row of wild sea-beet, type *W*, from seed collected near Wells-next-the-Sea, Norfolk, and (right) of Kleinwanzleben-E sugar-beet, to show the contrast between the prostrate leaves of wild beet and the upright leaves of sugar-beet. Photographed on 8 August 1956 by J. W. Blencowe. These plants were larger than the ones used for the experiment, because they grew from seed that germinated earlier (see text, p. 432).

By the end of the experiment, at harvest 3, the sugar-beet plants had produced about 40 per cent. more dry matter than the N and W types of wild beet, and about 80 per cent. more than type P (Table 1). The difference was mainly in dry weight of root, as might be expected because sugar-beet were bred for high root yield. Sugar-beet and wild type N had identical leaf-area indexes, so the higher yield of sugar-beet than of N was wholly due to its greater mean net assimilation rate in the second interval. At the other extreme, sugar-beet and type P wild beet had nearly the same E; here the greater yield of sugar-beet was due to its greater L. Evidently the superiority of sugar-beet in dry-matter production was attributable to its ability to combine a leaf-area index as high as the maximum of the wild beet types, with a high net assimilation rate which the wild beet types could equal only when the leaf-area index was much smaller.

The experiment here described and the earlier ones on sugar-beet and kale show that the sensitivity of mean net assimilation rate of a field crop to change in leaf-area index is a property of the plant that has great significance in relation to yield. We propose to call it the *leaf-density dependence* of net assimilation rate (symbol D). It can be assessed quantitatively, as above, by the regression coefficient of E on L, expressed as a percentage of E when $L = 1$.

The conclusion from the present experiment therefore, is that the development of sugar-beet from wild types by selection and breeding has not affected the intrinsic efficiency of the photosynthetic mechanism but has diminished its leaf-density dependence.

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The arrangement of Sepals and Petals in *Parnassia palustris* L.

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With five Figures in the Text

ABSTRACT

The aestivation of the calyx and corolla in 640 flower buds of *Parnassia palustris* L. from two sites in Britain is recorded.

Only two types of calyx arrangement are found. They are quincuncial, one being the mirror-image of the other and they continue the spiral from the bracteoles in the left or right direction respectively. The two types occur in almost equal numbers.

Twenty-two of the possible thirty-two corolla arrangements are found and there is a predominance of apotactous forms. The convolute corolla does not occur. The three commonest corolla types found in flowers with a right calyx are respectively the mirror-images of the three commonest corolla types in flowers with a left calyx. Corolla arrangements differing greatly from the six common types are rare. Although the completely eutopic corollas are not found, the commonest types are strongly eutopic and there is a highly significant tendency to eutopy in each sample as a whole. In flowers of each calyx type the overlaps between the two posterior petals and on the β-bracteole side of the flower are predominantly eutopic whereas the two overlaps on the α-bracteole side involving petal 9 are nearly always metatopic.

These observations are compared with those made on other species with variably imbricate pentamerous corollas and their significance is briefly discussed.

INTRODUCTION

TWO random collections of mature flower buds were made—a small one of about 160 from Mallaig, Inverness-shire, Scotland, in September 1957 (sample 1), and a large one of some 550 flowers from Claife Heights, Lancashire, England, in August 1958 (sample 2). The buds were preserved in 70 per cent. alcohol and later carefully dissected under a binocular microscope to determine the arrangement of sepals and petals. Although over 600 flower buds were dissected only two were found which were not pentamerous. These both had six sepals and were excluded from the samples subsequently analysed.

Obviously it was necessary to determine accurately the orientation of the flower, which is borne on a long, often twisted, pedicel in the axil of a radical leaf. There are two bracteoles, a basal α-bracteole which is similar to a foliage leaf and may bear further flowers in its axil, and the β-bracteole which is sessile, heart-shaped, and encircles the pedicel approximately half-way up. Above the β-bracteole the pedicel is unequally five-sided in section with one

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of the sepals inserted above each concave side (Fig. 1A). By tracing the concave faces of the pedicel up from the base it was definitely established that the posterior sepal was always the one above the second largest face. Thus, even though most of the flower buds were collected with only about 2 cm. of the pedicel attached to each, it was possible to orientate them correctly before dissection.

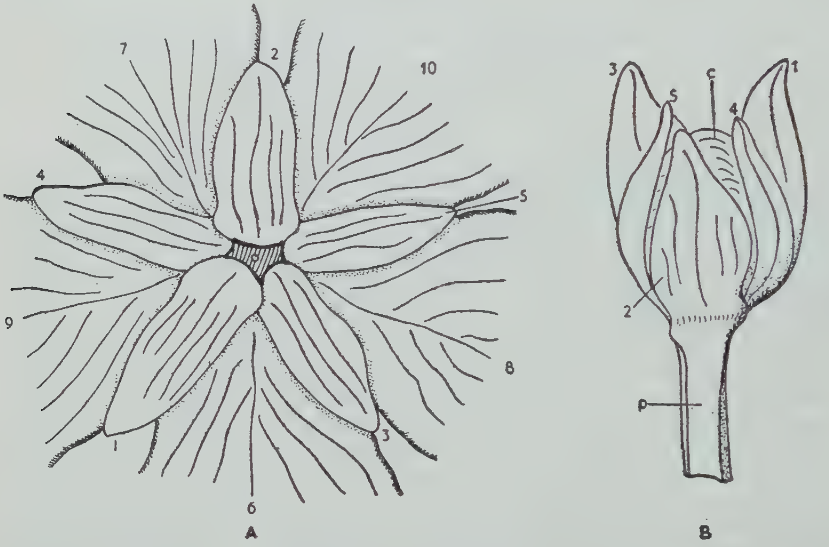

FIG. 1. *Parnassia palustris*. A, view from below of basal part of an open flower with the pedicel cut close to the insertion of the calyx. The sepals are numbered 1–5 in the left spiral sequence (when viewed from above). Sepal 1 is inserted above the largest concave face of the pedicel and the posterior sepal 2 has the two innermost sepals on either side of it. Petals numbered 6–10. B, lateral posterior view of mature flower bud with right calyx; 1–5, sepals; *c*, corolla; *p*, pedicel (Both × 5).

In many Dicotyledons the sepals tightly overlap each other at a very early stage in their development thus completely enclosing the rest of the flower. The calyx of *Parnassia* is different, with the distal parts of the lanceolate sepals projecting upwards, thus encircling but not enclosing the corolla which can always be seen as a white sphere from above (Fig. 1B). However, the broad bases of the sepals overlap and are inserted at slightly different levels round the top of the pedicel. The overlapping of the sepals is such that a $\frac{2}{5}$ spiral can be traced from the subtending leaf, through the two bracteoles and continuing from the lowest sepal inserted above the widest concave face of the pedicel to the highest sepal inserted above the narrowest face of the pedicel (Fig. 1A). Thus the arrangement of the calyx is always quincuncial with two sepals having both their edges outside (numbers 1 and 2), one sepal with one edge overlapping and the other overlapped (number 3), and two sepals with both edges overlapped (numbers 4 and 5). As this calyx arrangement was always found it provided an additional method of orientating the flower. The posterior sepal (number 2) was always identified as being the one

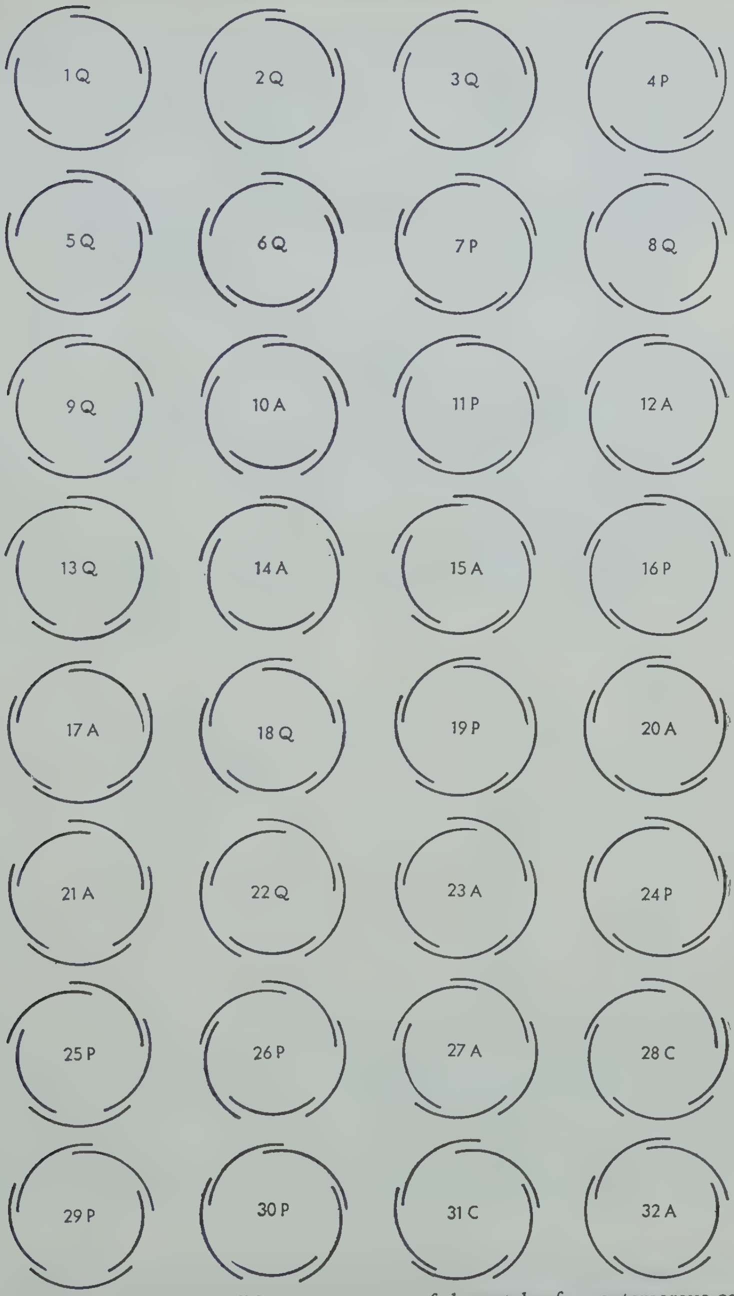

FIG. 2. Diagrams of the 32 possible arrangements of the petals of a pentamerous corolla. Q, quincuncial; P, paratactous; A, apotactous; C, convolute. (After Cunnell, 1958.)

with both its edges overlapping and having on either side of it the two sepals with both their edges overlapped (Fig. 1A).

Two types of this calyx arrangement were found, one with the spiral

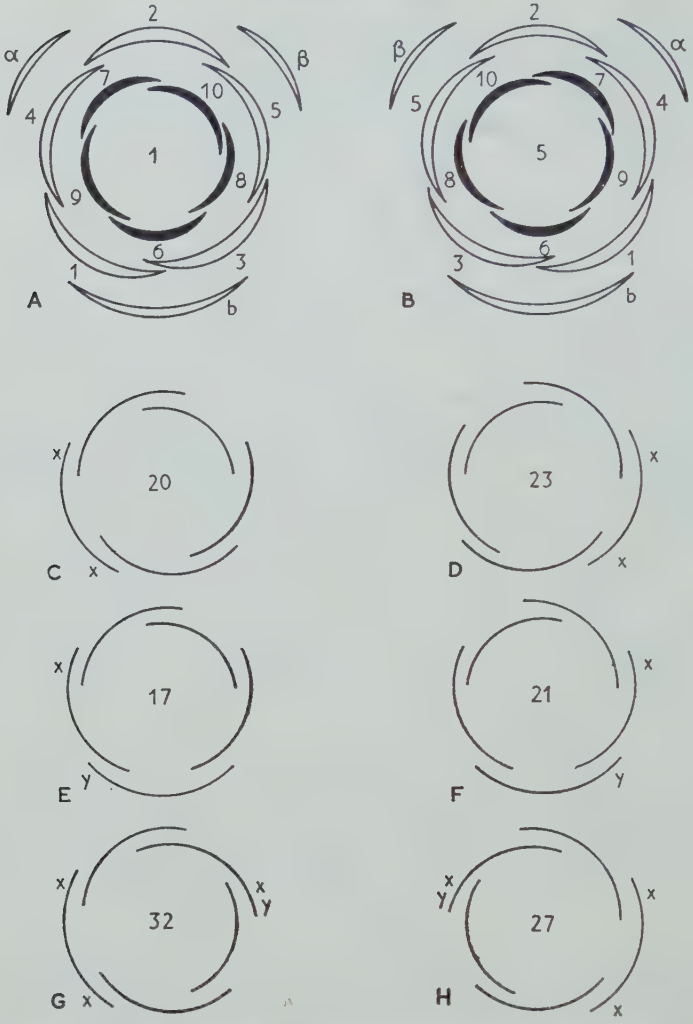

Fig. 3. A, diagram of floral envelopes of a right eutopic pentamerous flower; B, diagram of floral envelopes of a left eutopic pentamerous flower; C, commonest type of corolla arrangement found in right calyx flowers; D, commonest type of corolla arrangement found in left calyx flowers; E and G, other common corolla arrangements occurring in flowers with a right calyx; F and H, other common corolla arrangements occurring in flowers with a left calyx. *b*, subtending leaf; α, β, bracteoles; 1–5 sepals and 6–10 petals numbered in the $\frac{2}{5}$ spiral sequence; *x*, petal overlaps which differ from the eutopic ones in flowers of corresponding calyx type; *y*, petal overlaps which differ from those of the commonest corolla arrangement in flowers of the corresponding calyx type.

following a right-handed direction round the centre of the flower when seen from above and the other being the reverse or mirror-image of this with a left-handed spiral (Fig. 3 A, B). As dissection of both collections proceeded it became obvious that the two types of calyx occurred in almost equal numbers. Apparently the calyx type of any flower is purely a matter of chance. Therefore, to make direct comparisons easier, equal numbers of buds of each calyx type were dissected. Hence dissection of the smaller collection was continued until the corolla arrangements of 64 flowers with left calyces and 64 flowers with right calyces had been recorded. Similarly, for the larger sample 256 flowers of each calyx type were dissected.

Following Schoute (1935) and Cunnell (1958) the petals are numbered 6–10 in such a way that the spiral of the calyx can be continued through the corolla from the innermost sepal number 5. Thus the anterior petal is numbered 6 (Fig. 3 A, B).

THE COROLLA

It has already been pointed out (Cunnell, 1958) that with a circle of five petals there are $2^5 = 32$ possible arrangements of the petal overlaps. These can be put into four main groups, namely: ten quincuncial with two petals entirely inside, two entirely outside and one with one edge inside and the other outside; ten paratactous with one petal outside, an adjacent one wholly inside and the other three half overlapped; ten apotactous, similar to the paratactous arrangement but with the single inside petal not next to the outside petal; and two convolute with all five petals having one edge overlapping and the other overlapped.

TABLE 1

Main Corolla Types in each Sample

Main corolla types	Sample 1				Sample 2			
	Right calyx	Left calyx	Total	Total expected	Right calyx	Left calyx	Total	Total expected
Quincuncial	2	4	6	40	6	10	16	160
Paratactous	5	5	10	40	15	14	29	160
Apotactous	57	55	112	40	235	232	467	160
Convolute	0	0	0	8	0	0	0	32

The distributions of the four main corolla types set out in Table 1 show a marked similarity between the two samples and also between the flowers of the two calyx types in each sample. Further, there is a great discrepancy between these distributions and those expected if the overlapping of the petals were a matter of chance and all thirty-two possible arrangements were equally likely to occur. In both samples there is a preponderance of apotactous types, few quincuncial and paratactous arrangements occur and the convolute corolla is absent.

If overlapping of the petals is only a matter of chance it would be expected that the thirty-two arrangements possible in a pentamerous corolla would occur in a random sample with more or less equal frequency. That this is not so in *Parnassia* can be seen from the distributions for both samples given in Table 2 where the numbering of the thirty-two possible types is the same as that used previously for *Ranunculus repens* (Cunnell, 1958) (Fig. 2). The distributions of the two samples are strikingly similar; in all, only twenty-two types were found and these were very unequally represented. A further point of interest concerns the distribution of corolla types in flowers with right or left calyces. In sample 1 none of the corolla types found in flowers with right calyces occurred in flowers with left calyces and vice versa. This is almost true of sample 2 except for the few flowers with type 22 and 24 corollas where both types of calyx are found.

TABLE 2

Frequencies of all Possible Corolla Types found in each Sample

		Sample 1		Sample 2	
Corolla number*	Corolla type	Right calyx	Left calyx	Right calyx	Left calyx
1	Q	—	—	—	1
2	Q	—	—	—	—
3	Q	—	3	—	1
4	P	—	—	3	—
5	Q	—	—	—	—
6	Q	—	—	—	1
7	P	—	1	—	—
8	Q	1	—	3	—
9	Q	—	—	—	—
10	A	—	—	—	—
11	P	—	—	—	—
12	A	1	—	1	—
13	Q	—	—	—	—
14	A	—	—	—	—
15	A	—	1	1	—
16	P	—	—	—	1
17	A	15	—	56	—
18	Q	1	—	2	—
19	P	—	—	—	1
20	A	30	—	141	—
21	A	—	7	—	53
22	Q	—	1	1	7
23	A	—	29	—	139
24	P	1	—	2	1
25	P	—	4	—	11
26	P	—	—	—	—
27	A	—	18	—	40
28	C	—	—	—	—
29	P	3	—	9	—
30	P	1	—	1	—
31	C	—	—	—	—
32	A	11	—	36	—

* Numbering as used previously for *Ranunculus repens* (Cunnell, 1958); Q = quincuncial; P = paratactous; A = apotactous; C = convolute.

With both right and left calyx flowers the three most common corolla arrangements are apotactous and therefore account for the preponderance of this type over the other three shown in Table 1. In both samples approximately half the right calyx flowers had corolla type 20 (Fig. 3C) and type 23 corolla (Fig. 3D) was the commonest in flowers with left calyces. It is interesting to note here that the type 20 corolla is the mirror-image of corolla type 23. Therefore, as these are strictly associated with right and left calyces respectively, it follows that the two commonest perianth arrangements found in these *Parnassia* flowers are mirror-images of each other.

Corolla types 17 and 32 are also well represented in flowers with a right calyx. These are very similar to the predominant type 20 and each only differs from it at one overlap position. A similar situation is found in flowers with a left calyx where the fairly common corolla types 21 and 27 only differ from the predominant corolla type 23 in one position. The number of corollas which differ in varying degrees from the commonest two types set out in Table 3 shows that corollas differing from the normal ones at more than two positions are very rare and did not appear at all in the smaller sample 1. In sample 1 13 per cent. and in sample 2 only 10·5 per cent. of the total overlaps did not conform to those of corolla types 20 and 23.

TABLE 3

Frequencies of Corollas Differing by Various Degrees from the Predominant Types (type 20 for flowers with a right calyx and type 23 for flowers with a left calyx)

Number of positions corolla differs from predominant type	Number of corollas			
	Sample 1		Sample 2	
	Right calyx	Left calyx	Right calyx	Left calyx
0	30	29	141	139
1	28	27	99	101
2	6	8	15	14
3	0	0	0	1
4	0	0	0	1
5	0	0	1	0

Corolla types 17 and 32, which are confined to flowers with right calyces, are respectively the mirror-images of corolla types 21 and 27 which are only found in flowers with left calyces (Fig. 3 E–H). It is only at the 9–6 position that type 17 corolla differs from the predominant type 20 for right calyx flowers and it is at this same 9–6 position that type 21 corolla differs from the usual type 23 corolla of flowers with left calyces. Similarly it is at the position of overlap between petals 8 and 10 that type 32 differs from type 20 in flowers with right calyces and type 27 differs from type 23 in flowers with left calyces.

Clearly, taking each sample as a whole, there are two predominant types of corolla configuration, intimately associated with the calyx arrangement and mirror-images of each other. Most of the other corolla types found differ only slightly from the main ones and their 'abnormal' overlaps are far commoner in some positions than in others. Table 4 shows that in flowers with either right or left calyces 'abnormal' overlaps are rare between petals 6 and 8, 10 and 7, and 7 and 9. Conformity with the 'normal' is, however, much less rigid in the 8–10 and 9–6 positions.

TABLE 4

Frequencies of 'Abnormal' Arrangements at the Five Petal Overlap Positions

	Number of 'Abnormal' overlaps			
	Sample 1		Sample 2	
Overlap positions	Right calyx	Left calyx	Right calyx	Left calyx
6–8	2	1	5	10
8–10	17	26	51	54
10–7	2	3	7	3
7–9	1	2	5	2
9–6	18	11	66	66

The method of analysis of corolla aestivation devised by Schoute (1935) and used on the flowers of *Ranunculus repens* (Cunnell, 1958) may also be applied to these samples of *Parnassia* flowers. For a flower with a right calyx there is one arrangement of the corolla which continues the spiral of the sepals. This is the quincuncial type 1 which is described as eutopic (Fig. 3A). Similarly, type 5 is the eutopic corolla for a flower with a left calyx (Fig. 3B). All other corolla configurations can be designated as $\frac{4}{5}$ eutopic to $\frac{0}{5}$ eutopic (i.e. metatopic) according to the number of overlaps which are the same as those of the completely eutopic arrangement.

Table 5 shows the frequencies of flowers with different degrees of eutopy and indicates that they are highly significantly different from the frequencies expected if the corolla types were merely random ($P = < 0{\cdot}001$). Although this is mainly due to the large number of $\frac{3}{5}$ eutopic flowers, a category to which the commonest types 20 and 23 belong, there is also a deficiency of $\frac{5}{5}$, $\frac{1}{5}$, and $\frac{0}{5}$ eutopic flowers.

TABLE 5

Frequencies of Corollas Showing Different Degrees of Eutopy

	Sample 1			Sample 2		
	Right calyx	Left calyx	Frequency expected in each calyx group	Right calyx	Left calyx	Frequency expected in each calyx group
$\frac{5}{5}$ Eutopic	0	0	2	0	0	8
$\frac{4}{5}$ Eutopic	15	8	10	59	53	40
$\frac{3}{5}$ Eutopic	34	34	20	151	153	80
$\frac{2}{5}$ Eutopic	13	19	20	41	49	80
$\frac{1}{5}$ Eutopic	2	3	10	5	1	40
$\frac{0}{5}$ Eutopic	0	0	2	0	0	8

The total number of eutopic and metatopic overlaps is shown in Table 6. For both right and left calyx flowers there are significantly more eutopic than metatopic overlaps ($P = < 0{\cdot}001$). Thus although the completely eutopic corolla types themselves are not found at all there is a marked tendency to eutopy in each sample as a whole.

TABLE 6

Total numbers of eutopic and metatopic petal overlaps

	Sample 1				Sample 2			
	Right calyx	Left calyx	Total	Total expected	Right calyx	Left calyx	Total	Total expected
Eutopic	190	175	365	320	776	770	1,546	1,280
Metatopic	130	145	275	320	504	510	1,014	1,280

At each position of overlap two arrangements are possible and for a flower with a given type of calyx one of these will be eutopic and the other metatopic. Therefore the analysis may be carried further by determining the extent of eutopy at each of the five positions where adjacent petals overlap.

The frequencies given in Table 7 show that the two samples are very similar. At the 6–8 and 10–7 positions the overlaps are almost entirely eutopic and at the 7–9 position they are nearly always metatopic. The 8–10 position is also strongly eutopic and the 9–6 position is metatopic. At every position the frequency distribution of the two types of overlap is statistically highly significantly different from that expected were they due purely to chance ($P = < 0{\cdot}001$). This is made clear in Fig. 4 which also illustrates how the distribution of overlaps of the petals in flowers with one type of calyx is the mirror-image of the other.

TABLE 7

Distribution of Eutopy and Metatopy at the Five Positions of Petal Overlap

Sample 1

	Right calyx		Left calyx		Totals	
Petal overlap	Eutopic	Metatopic	Eutopic	Metatopic	Eutopic	Metatopic
6–8	62	2	63	1	125	3
8–10	47	17	38	26	85	43
10–7	62	2	61	3	123	5
7–9	1	63	2	62	3	125
9–6	18	46	11	53	29	99

Sample 2

	Right calyx		Left calyx		Totals	
Petal overlap	Eutopic	Metatopic	Eutopic	Metatopic	Eutopic	Metatopic
6–8	251	5	246	10	497	15
8–10	205	51	202	54	407	105
10–7	249	7	253	3	502	10
7–9	5	251	2	254	7	505
9–6	66	190	67	189	133	379

DISCUSSION

As far as can be discovered from the literature aestivation in *Parnassia palustris* has not been investigated previously. It does, however, provide an interesting comparison with other species having actinomorphic, pentamerous

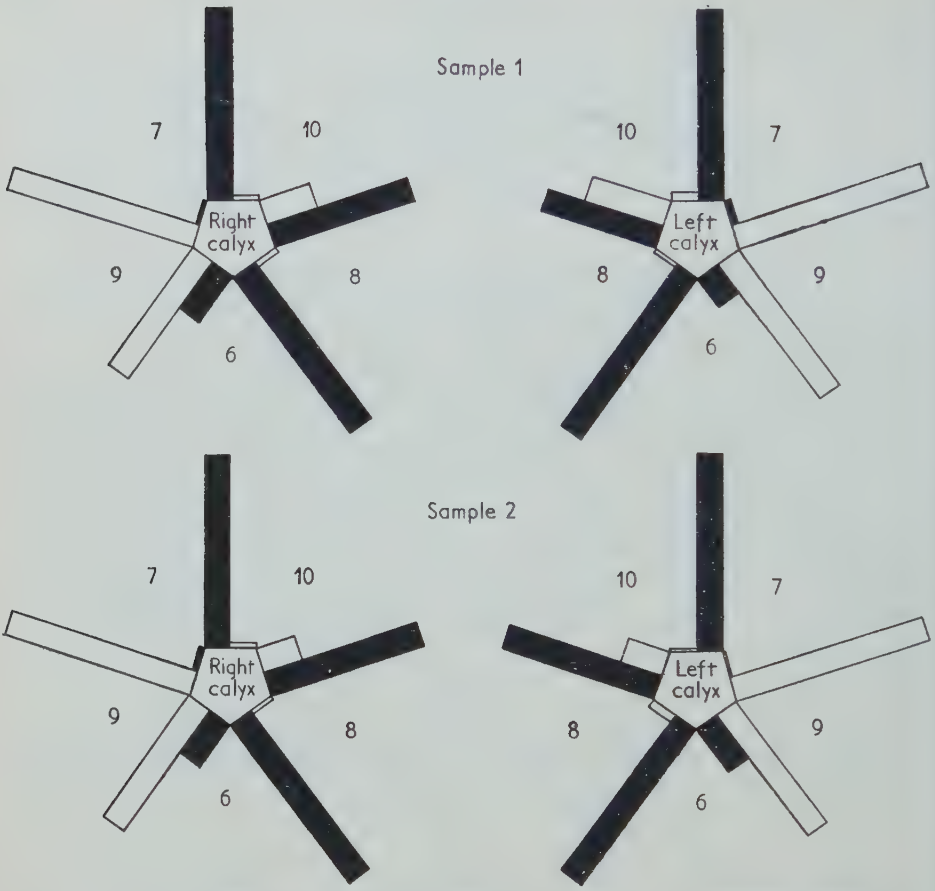

FIG. 4. Petal overlap diagrams of *Parnassia* flower buds. The two columns based on each side of the pentagon represent the frequencies of the two possible overlaps between adjacent petals, the positions of which are numbered 6–10. Thus in most flowers petals 7 and 8 overlap petal 10. Eutopic overlaps black, metatopic overlaps white. Scale of diagram for Sample 2 is a quarter that of diagram for Sample 1.

flowers showing variably imbricate aestivation. The two samples used in this investigation were of different sizes and collected in different years from sites over 200 miles apart. In spite of this the analysis reveals a striking similarity between the two samples and for purposes of discussion it is hardly necessary to separate them.

The number of perianth parts in *Parnassia* appears to be very stable. All the flowers had five petals and only two out of more than 600 that were dissected had six sepals. This uniformity was much greater than that found in a similar-sized sample of *Ranunculus repens* where twenty-nine had an abnormal number of petals or sepals (Cunnell, unpublished).

The arrangement of the sepals is always quincuncial and apparently it is a matter of chance whether the calyx of any one flower shows a left or right spiral. This agrees with the situation found in *Ranunculus* spp. (Riley, 1923; Cunnell, 1958) where the second oldest sepal is the posterior one. However, in *Parnassia* the calyx is entirely stable and no other arrangement of the sepals was found. The sepals are clearly arranged in a $\frac{2}{5}$ spiral. This is shown, not only by the overlapping of the sepals, but also by their insertion at different levels at the top of the pedicel and sometimes by size differences. The oldest sepal, number 1, is inserted at the lowest level and is the largest, whereas the youngest sepal, number 5, is at the highest level and is often the smallest (Fig. 1A).

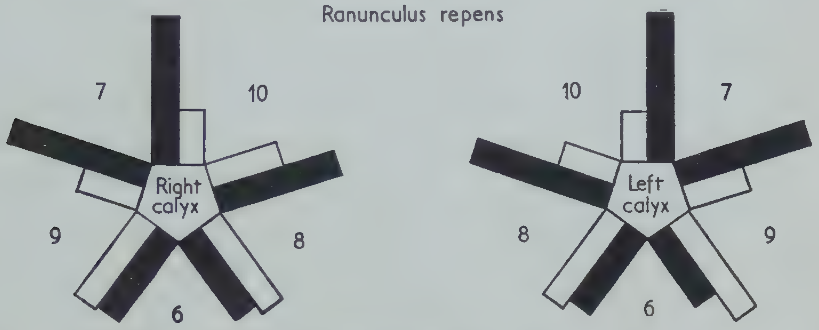

Fig. 5. Petal overlap diagrams of lateral flower buds of *Ranunculus repens* based on data given in Cunnell, 1958. Scale of diagram is one-fifth that of diagram for Sample 1 in Fig. 4. For explanation see Fig. 4.

In the lateral flower buds of *Ranunculus repens* the two prophylls (bracteoles) are often similar in size and are inserted at the same level on the pedicel. The two bracteoles of *Parnassia* are very different from each other, the α-bracteole being radical and leaf-like whereas the heart-shaped β-bracteole is inserted half-way up the pedicel. Although no developmental studies have been undertaken it can be presumed that as a flower develops in the axil of a radical leaf the β-bracteole will be the first formed lateral structure on the primordium. This would suggest that it is the position at which the β-bracteole appears that ultimately determines the spiral direction of the calyx. If, when looking at the primordium from above, the β-bracteole appears on the left of the anterior-posterior line then the calyx will have a right spiral. If it appears on the right then the flower will have a calyx with a left spiral (Fig. 3 A, B).

The corolla is much more variable and twenty-two of the possible thirty-two overlapping arrangements were found. In this respect *Parnassia* is not so variable as *Ranunculus repens*, two samples of 320 flowers of which contained all thirty-two corolla types (Cunnell, 1958). In *R. repens* the quincuncial corolla arrangement is the commonest whereas in *Parnassia* 89·5 per cent. of the flowers had apotactous corollas. Nevertheless, the striking correlation of calyx type and corolla is shown. Twelve corolla types were found in flowers with a right calyx and, with the small exception of one flower each with corolla types 15, 22, and 24, these were different from the eleven corolla arrangements in flowers with a left calyx. The mirror-image relationship shown by the two calyx types is repeated in the corollas. The three commonest corollas of flowers with a right calyx, types 20, 17, and 32 are respectively the mirror-images of types 23, 21, and 27 which are the ones most commonly found in the left calyx flowers (Fig. 3 C–H).

This relationship is by no means as rigid in *Ranunculus repens* where most of the corolla types are found, although often unequally, in flowers with left and right calyces. It is interesting to find that of eleven flowers with type 20 corolla nine had a right calyx and that nine out of twelve flowers with type 23 corolla had a left calyx. Hence, in so far as these corolla arrangements are found in *R. repens* they also show a relationship with the calyx types although they are not mutually exclusive as in *Parnassia*.

Clearly, corolla types 20 and 23 are the predominant ones in *Parnassia* and, as Table 3 shows, the occurrence of others which differ greatly from these is very rare. Differences are found mainly at two positions, petal 8 being overlapped by petal 10 and petal 6 overlapping petal 9. At the other three positions very few abnormal overlaps are found.

Corolla types 20 and 23 differ respectively from the eutopic types 1 and 5 only in that petal 9 is outside instead of being overlapped by petals 6 and 7 (Fig. 3 A–D). Further, the next commonest types 17 and 21 only differ from types 20 and 23 respectively in that petal 6 overlaps petal 9 and therefore they are even more eutopic. Thus, although the predominant types are apotactous and not quincuncial they are strongly eutopic and account for the significant tendency to eutopy shown by each sample as a whole. Types 32 and 27 which are also well represented have the metatopic position of petal 9 outside the neighbouring ones and also the metatopic arrangement of petal 10 over petal 8. However, these two types are not sufficiently numerous to upset the clear picture of distribution of eutopy and metatopy shown in Fig. 4. The overlaps on the median line between the two posterior petals 10 and 7 are predominantly eutopic as also are those at the 10–8 and 8–6 positions on the β-bracteole side of the flower. The overlaps on the α-bracteole side of the flower involving petal 9 are predominantly metatopic. Thus, although there is an undeniable retention of spiral influences in the corolla as a whole it appears that other factors are operating in such a way that the two sides of the flower are very different. Nothing is known of these factors affecting the symmetry but it could in some way be related to the great difference between the position and shape of the two bracteoles.

In the lateral flowers of *Ranunculus repens*, where the bracteoles are similar in shape and position, eutopy is strong at the three posterior positions and absent at the overlaps involving the anterior petal (Fig. 5, based on data from Cunnell, 1958). Thus there is an apparent impress of zygomorphy on a basically spiral condition, whereas in *Parnassia* the factors upsetting the spiral lead to a condition of asymmetry.

Schoute (1935) in his long review on aestivation has dealt in some detail with variably imbricate corollas. Although his observations in most cases are based on small samples he divides the species investigated into several categories. Some, such as *Ranunculus lingua* show a tendency to eutopy at all five positions whereas in others, like *Rosa canina*, this tendency is absent at one or more edges. The third category which includes *Ranunculus acris* has an evenly distributed chance aestivation. His last group includes species where both eutopy and metatopy are found. Clearly *Parnassia* belongs here but it appears to be unlike any species investigated by Schoute although coming nearest to a member of the Crassulaceae, *Cotyledon gibbiflorus*. As in *Parnassia* this species is eutopic at the 6–8 and 10–7 positions and metatopic at the 9–6 position but at the 7–9 position it is strongly eutopic and its 8–10 overlap is weakly metatopic. He found a tendency to metatopy at the 8–10 position in several species and suggested that this may be due to the influence of the β-bracteole which is situated more or less beneath petal 8. This idea cannot be applied to *Parnassia* as petal 8 shows a strong eutopic tendency at both its edges. In fact it is petal 9 which displays metatopy at both its edges and the factors responsible for this are completely unknown.

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The Infection of Pine stumps by *Fomes annosus* and other fungi

BY

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ABSTRACT

A study of the infection biology of stump-colonizing fungi has shown that certain species, for instance *Fomes annosus*, *Peniophora gigantea*, *Stereum sanguinolentum*, and several causing blue-stain, initiate infection of fresh stumps by means of air-borne spores which colonize the cut surface. The incidence of natural infection is reduced considerably by applying creosote to the surface immediately after felling. High resin content of stumps is correlated with increased resistance to infection. Marked seasonal variation in the incidence of stump infection by *F. annosus* and *P. gigantea* was observed. The spore content of the air, and competition between fungi in stumps, are considered to be important factors affecting this variation.

Initial colonization of stump roots usually proceeds from the body of the stump. Invasion of roots by saprophytes present in the soil or litter also occurs but only many months after felling.

Crowns of plantation pines may bear many spores of stump-colonizing fungi, including *F. annosus*. Under the conditions described, the proportion of stump infections ascribable to rain-splash-borne spores was small compared with that caused by wind-borne spores.

INTRODUCTION

THE colonization by fungi of stumps remaining after a stand of trees has been felled is of interest to both the forest pathologist and the mycologist.

Petch (1921), working in Ceylon, realized the important role that stumps might play in the spread of root disease in plantation crops. He put forward the hypothesis that air-borne spores of parasites such as *Fomes lignosus*, *F. noxius*, and *Ustulina zonata* infect the exposed surfaces of jungle stumps, and suggested that these fungi then grew down the stumps into the roots. Subsequently, they infected roots of young plantations crops, either directly through root contacts, or by free mycelial spread through the soil. Unfortunately, no experimental evidence was produced to support this hypothesis. In Malaya, Napper (1934) considered this mode of initial entry to be of secondary importance in the development of root disease in young rubber plantations. His control measures were based on the assumption that the most important source of inoculum for root infection lay in the old stump roots left after clearing jungle trees. Since these measures were successful, no experiments were conducted to assess the importance of stump infection

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by air-borne spores. Leach (1937, 1939), working on tea plantations in Nyasaland, reported that all attempts to infect stumps with basidiospores of *Armillaria mellea* were unsuccessful.

The first demonstration of aerial infection of stumps was provided by Rishbeth (1951*a*, 1951*b*), working on root disease caused by *Fomes annosus* in East Anglian pine plantations. Many observations of naturally infected stumps were explicable only on the assumption that basidiospores had infected the exposed stump surface. Experimental inoculations of freshly exposed stump surfaces were successful. Rishbeth traced the growth of the fungus from the stump surface into the roots, and observed infection of adjacent healthy trees through root contacts. Spreading in this manner from a single stump, the fungus often caused considerable disease gaps. Further evidence in support of this stump infection hypothesis was provided by treating stump surfaces in various ways immediately after felling. In one experiment, only 4 per cent. of stumps which had been creosoted were infected with *F. annosus*, whereas 36 per cent. of normally exposed stumps contained the fungus. If the fungus always entered stumps from the soil or roots, no measure of control would be expected by creosoting. Later observations on this experiment showed that the incidence of tree killing was clearly related to the proportion of stumps earlier infected by *F. annosus* (Rishbeth, 1957).

As a result of this work, considerable interest has developed on the concept of stump infection by *F. annosus*, particularly by Scandinavian workers. One objection raised is that this mode of infection cannot always satisfactorily explain the spread of the fungus in Swedish forests (Rennerfelt, unpublished, cited by Molin, 1957), and the hypothesis generally favoured is that infection of standing trees proceeds directly from the soil. The present situation can be summed up by a comment made by Garrett (1956), reviewing control measures of root disease in plantation crops, who states 'it is therefore futile to debate the probability of one hypothesis or the other holding true for any particular area when the question can so simply be tested through the methods devised by Rishbeth'.

Little information exists concerning the succession of fungi causing decay of pine stumps. Rishbeth (1950, 1951*a*) has outlined the probable course of succession in stumps rotted by *F. annosus*, paying particular attention to interaction of this fungus and *Trichoderma viride*, which frequently replaces the former. During studies on the ecology of the insect fauna of pine stumps, Wallace (1953) suggested that fungal decay probably plays some part in determining the distribution of insects. Recently, Käärik and Rennerfelt (1957), working on the fungal flora of spruce and pine stumps in Swedish forests, distinguished between 'forest rot fungi', such as *A. mellea* and *F. annosus* which survive in the stumps of infected trees, and 'stump fungi', which they suppose infect the stump surface by means of air-borne spores. Belonging to the latter group are several members of the Agaricaceae, Polyporaceae, and Thelephoraceae. Samples taken from stumps of different ages showed that the frequency of occurrence of several species was related to stump age. The moisture and resin content of stumps are considered to influence the course of natural decay.

The present paper describes investigations on the infection biology of fungi colonizing pine stumps. Most of the work was carried out in Forestry Commission pine plantations in East Anglia, within the region known as Breckland. These plantations were established by the Forestry Commission from 1922 onwards, and only in recent years have they been subjected to extensive thinning. *Pinus sylvestris* L. and *P. nigra* var. *calabrica* Schneid. are most commonly planted, and the present study has been concerned with stumps of these species. In recent years, *F. annosus* has caused serious damage in these plantations, and in 1952, as a result of the work of Rishbeth (1950, 1951*a*, 1951*b*, 1952), the Forestry Commission initiated large-scale creosoting for first-thinning stumps at sites where *F. annosus* attacks were likely to be severe. In 1954, the treatment was extended to all thinning operations.

GENERAL METHODS

Sampling methods. Occasionally, complete stumps were extracted in order to obtain the fullest information about the fungi present. However, since this was unnecessary for many recording purposes, several more convenient sampling techniques were employed.

Bark samples. This technique was devised by Rishbeth (1950) for his studies on *F. annosus*. Bark is removed from the stump, wrapped in newspaper and, after wetting with tap water, incubated for 4–5 days at room temperature. The presence of *F. annosus* is indicated by the development of characteristic conidia. The method is not restricted to *F. annosus* since several other stump-colonizing fungi, for instance *Peniophora gigantea* and some species causing blue-stain, also produce characteristic spores after incubation. A few fungi which do not form conidia can be identified by their mycelium, examples being *Polystictus abietinus* and *Hypholoma fasciculare*, which frequently develop characteristic mycelial strands between the bark and wood.

As bark samples are relatively small and quickly collected, they have been widely used in survey work. The major limitation of the technique is that sampling must be delayed for at least six months from the time of felling, to allow time for the fungi present in the wood to colonize the periphery of the stump. Also, although a stump may be colonized by a particular fungus in the central regions, other species may be present in the periphery.

Wood samples. A section 4–6 cm. thick was cut from the top of the stump, wrapped immediately in newspaper and then incubated in the same manner as a bark sample. After 4–6 days, the presence of certain fungi, for instance *F. annosus*, *P. gigantea*, *H. fasciculare*, *P. abietinus*, and several blue-stain fungi, e.g. *Ceratocystis* spp., was generally indicated by the development of conidia or mycelium from the wood. With the aid of a binocular dissecting microscope, the distribution of fungi over the cut surface was mapped.

Experimental inoculation of the stump surface with basidiospores of

F. annosus and *P. gigantea* showed that reliable samples could be taken after 4 weeks. When investigating natural infection, at least 8 weeks were allowed to elapse between felling and sampling. A period of 12 months was considered to be the maximum permissible for studies on early stages of infection: if sampling is delayed further, fungi originally occupying the upper stump regions may have become replaced by competitors.

To compare the suitability of wood and bark samples for recording the frequency of infection, stumps of different ages were sampled simultaneously by both methods. *F. annosus* and *P. gigantea* were the species recorded, since they were generally the most frequent early colonists. The results of the samplings are given in Table 1.

TABLE 1

Comparison of Two Sampling Methods for recording the Frequency of Stump Infection by F. annosus *and* P. gigantea: *the Percentage of Samples Infected*

		Age of stumps (months)		
		3	6	12
	No. of samples	68	60	40
	Sample type			
F. annosus	Bark	3	25	15
	Wood	41	60	28
P. gigantea	Bark	7	45	83
	Wood	40	60	95

The results indicate clearly that wood samples are more reliable than bark samples for recording infection by these fungi up to 1 year after felling. Agreement between data derived from both sample types increases with age of stump.

Root samples. Stump roots were exposed for the greater part of their length and severed close to the body of the stump. They were washed free of adhering soil and loose bark scales. Each root was split longitudinally and the halves were incubated and examined in the manner previously described.

Isolation of fungi. It was necessary to obtain pure cultures of several stump-inhabiting fungi, both for identification and experimental purposes. Since most of the basidiomycetes colonizing stumps produce sporophores, isolates could be obtained from sporophore tissue or basidiospores. Isolations from infected wood were made by transferring slivers of wood aseptically on to the agar surface. Because of heavy bacterial contamination, it was often difficult to obtain pure cultures from very decayed stump wood unless the agar medium was acidified by adding 0·1 ml. of 2·5 N lactic acid to the agar at the time of pouring (Rishbeth, 1950). Isolates of several fungi imperfecti and blue-stain fungi were obtained by transferring conidia, which grew out of infected wood after incubation in a moist container for several days, on to 2 per cent. malt agar.

List of Species

The following is a list of the more common fungi found on pine stumps. Fruit-bodies of all the basidiomycetes and ascomycetes have been observed on stumps in various stages of decay.

Basidiomycetes

Armillaria mellea (Vahl) Quél.
Clitocybe aurantiaca (Fr. *ex* Wulf) St.
Collybia maculata (Alb. *et* Schw.) Fr.
Flammula sapinea Fr.
Fomes annosus Fr.
Hypholoma fasciculare (Huds.) Fr.
Marasmius spp.
Paxillus atrotomentosus (Batsch) Fr.
P. involutus Batsch.
Peniophora gigantea (Fr.) Massee.
Pholiota flammans Fr.
Pleurotus mitis (Pers.) Fr.
Polyporus adiposus B. and Br.
P. amorphus Fr.
P. sulphureus (Bull.) Fr.
Polystictus abietinus (Dicks.) Fr.
P. versicolor (Linn.) Fr.
Sparassis crispa (Wulf.) Fr.
Stereum sanguinolentum Alb. *et* Schw.
Tricholoma albo-brunneum (Pers.) Fr.
T. rutilans (Schaeff.) Fr.

Ascomycetes

Biatorella resinae (Fr.) Mudd.
Ceratocystis minor (Hedge.) Hunt.
C. piceae (Münch) Bakshi.
C. pilifera (Fr.) Moreau.
Ophionectria cylindrospora (Sollman) Berl. *et* Vogl.

Phycomycetes

Mucor ramannianus Möll.
Mucor spp.

Fungi Imperfecti

Aspergillus spp.
Botrytis cinerea Pers.
Cladosporium herbarum Link.
Fusarium spp.
Gliocladium viride Matruchot.
Gliocladium spp.
Graphium spp.
Leptographium lundbergii Lagerberg *et* Melin.
Penicillium spp.
Pullularia pullulans (de Bary *et* Low) Berkh.
Paecilomyces elegans (Corda) Mason *et* Hughes *apud* Hughes 1951.
Trichoderma viride Pers. *ex* Fr.

Infection of Stump Surface by Spores

Natural Infection

Stumps of healthy 15-year-old pines were sampled at intervals after felling. After incubation, the samples were examined for the presence of fungi. The results are given in Table 2.

Under natural conditions, infection of the stump surface may occur almost immediately after felling. The development of small, relatively isolated colonies in the samples suggested that infection was initiated by single spores.

TABLE 2

Natural Infection of Stumps: the Average Number of Colonies on Wood Samples removed at Intervals after Felling

	Interval between felling and sampling			
Fungus	0	1 hour	3 hours	5 days
P. gigantea	0·3	2·3	3·5	>10
Blue-stain fungi	0·3	1·0	1·8	1·0
F. annosus	0	0	0·8	1·8

Further evidence of natural infection was obtained by cutting off serial sections from 4-week-old stumps. Out of 126 stumps sampled, 120 contained *P. gigantea*, 39 various blue-stain fungi, for instance *Ceratocystis* spp., 24 *S. sanguinolentum* and 20 *F. annosus*. These fungi were present only in the upper 8–10 cm. of the stumps, suggesting that infection was initiated at the cut surface.

A preliminary experiment was set up to test a method of excluding airborne spores from stump surfaces. Healthy 20-year-old pines were felled and the stumps were immediately covered with sheets of moisture-proof 'Cellophane' secured to the stumps by rubber bands: as a control, a further series of stumps was left exposed. After 4 weeks, wood samples showed that of the 10 covered stumps *P. gigantea* was present in 2 and *F. annosus* in 1, whereas 9 of the uncovered stumps contained *P. gigantea* and 5 *F. annosus*. The results suggested that this technique might be employed in experiments where it was important to exclude aerial infection. The major limitation is that if stumps are covered for periods greatly exceeding 4 weeks, several moulds, in particular *T. viride*, develop on the stumps, probably as a result of an increased relative humidity at the stump surface. The effect of applying creosote to stumps, in reducing the incidence of infection, will be discussed later (p. 464).

A factor which may affect the incidence of stump infection is the resin content of stumps. Immediately after a healthy tree is felled, resin begins to exude from the stump surface. This process often continues for several weeks, resulting in a 'resin crust' on the surface and an accumulation in the wood at the top of the stump. Some evidence for the effect of resin was obtained from 4- and 5-year-old stumps, which are usually much decayed and colonized by a large number of fungi. Occasionally however, stumps of this age were found with healthy wood from which it was impossible to isolate fungi. The tops of such stumps had a very high resin content, which had apparently effectively sealed the stump against fungal invasion.

Further evidence concerning the effect of resin was obtained from trees which formerly had double stems, one having been removed during an earlier thinning. The 'stumps' of these trees are organically connected with the remaining stem, and exhibit resin flow for several months from the time of felling. The quantity of resin accumulating in the 'stump' appears to be related to the vigour of the remaining stem. If this is of a 'suppressed' tree, resin accumulation may be less than that of normal stumps; on the other hand, if the remaining stem is of a 'dominant' in the tree canopy then the resin accumulation often greatly exceeds that of normal stumps. Although 'double-leaders' are rather uncommon in the plantations, it was possible to find 'stumps' of a few differing greatly in their resin content and varying in age between 12 and 18 months. A total of 22 'stumps' were sampled and only 2 were colonized by fungi, the species being *P. gigantea* and *Leptographium lundbergii* respectively. It was significant that the two infected 'stumps' were attached to markedly suppressed stems, and that the quantity of accumulated resin was small. The absence of fungi in other 'stumps' was associated with a high resin content. Possibly, resin in large quantities acts by inhibiting spore germination and growth through the wood.

The period during which a stump remains susceptible to infection at the surface is probably limited by the formation of a 'crust' of exuded resin. This 'crust' often completely covers the surface after 3–4 weeks, and may act as a mechanical barrier against fungal invasion.

Experimental Infection

The observations described so far, and many other samplings, have shown that the fungi occurring most frequently in stumps shortly after felling are *P. gigantea*, *F. annosus*, *S. sanguinolentum*, and various fungi causing blue-stain.

An experiment was set up in a previously unthinned plantation of 20-year-old Scots pine to determine the ability of spores of different stump-inhabiting fungi to colonize new stumps. Immediately after felling, one series of stumps was inoculated with spore suspensions and then covered with 'Cellophane' sheets. Another series was inoculated with the same suspensions but left uncovered, while a third series was covered but not inoculated. Suspensions were counted by means of a haemocytometer and brought to a concentration of *c.* 1×10^4 spores per ml. Inoculations were made by spraying the stump surface with spore suspensions from an atomizer. After 4 weeks, the stumps were sampled by sawing off wood samples and these were incubated as already described. In addition, attempts were made to reisolate the fungi by transferring wood chips from the samples to malt agar. The results obtained are given in Table 3.

A striking feature of these results was the failure of spores of *P. abietinus*, *P. amorphus*, and certain Agaricaceae, which frequently inhabit older stumps, to initiate infection of fresh stumps. On the other hand, the remaining basidiomycetes and the three blue-stain fungi (*Ceratocystis* spp., *L. lundbergii*) showed a high incidence of infection. A few of the covered controls became infected, probably as a result of natural infection. The effect of covering stumps was most pronounced in those inoculated with *G. viride* and *T. viride:* the increased relative humidity at the surface of covered stumps probably favoured their development. There was no significant difference between the frequencies of infection of covered and uncovered stumps by the basidiomycetes or

TABLE 3

Experimental Infection of Stumps by Various Fungi: the Number of Stumps (out of 10) Infected 4 Weeks after Inoculation

Fungus	Inoculum	Stump treatment: Inoculated/ Covered	Inoculated/ Uncovered	Covered controls
Fomes annosus	Basidiospores	9	8	0
Hypholoma fasciculare	,,	0	0	0
Paxillus atrotomentosus	,,	0	0	0
Peniophora gigantea	,,	10	10	3
Polyporus amorphus	,,	0	0	0
Polystictus abietinus	,,	0	0	0
Stereum sanguinolentum	,,	9	7	0
Tricholoma rutilans	,,	0	0	0
Ceratocystis pilifera	Ascospores	10	10	1
C. minor	,,	10	10	0
Gliocladium viride	Conidia	6	1	0
Leptographium lundbergii	,,	10	10	2
Trichoderma viride	,,	8	1	4

blue-stain fungi, suggesting that under the experimental conditions relative humidity was not limiting spore germination or subsequent infection.

A further experiment was designed to test the ability of a few fungi to colonize stumps of trees varying in vigour. Two types of tree were selected, (1) healthy dominants with well-developed crowns and an average basal stem diameter of 13·4 cm., and (2) heavily suppressed trees, whose crowns were still living but very poorly developed, with an average basal stem diameter of 6·7 cm. Stumps of each type were inoculated with spore suspensions immediately after felling, and then covered with 'Cellophane'. The stumps were sampled after 8 weeks and the results are shown in Table 4.

TABLE 4

Infection of Stumps belonging to Trees Varying in Vigour: the Number of Stumps (out of 10) infected 8 weeks after Inoculation with Spore Suspensions

Fungus	Stumps belonging to: Dominant trees	Suppressed trees
F. annosus	9	9
H. fasciculare	0	0
L. lundbergii	10	10
P. gigantea	9	9
P. abietinus	0	0
S. sanguinolentum	8	9

The resin content of suppressed stumps was lower than that of dominants, as evidenced by the quantity exuding from the cut surface after felling; in some instances no resin exuded. However, the failure of *H. fasciculare* and *P. abietinus* to infect these stumps suggested that factors other than the resin content of wood were operative in determining the selectivity of fresh stumps towards fungal invasion. The results for the other fungi suggested that the susceptibility to infection of stumps of dominants was not markedly different from that of stumps belonging to less vigorous trees.

More definite evidence of the effects of high resin content was obtained from experiments using 'double-leader' trees. Each tree branched just above ground level and both stems had approximately equal diameters near the point of branching. One stem was removed from each tree and the 'stumps' were inoculated with spore suspensions. As a control, a series of normal stumps was inoculated with the same fungi. The stumps were sampled after 3 months, and in each case the amount of resin which had accumulated was noted. The results are given in Table 5.

TABLE 5

Experimental Infection of 'Stumps' of Double-stemmed Trees by Various Fungi

	'Stumps' of double-leaders		Normal stumps	
Fungus	No. inoculated	No. infected	No. inoculated	No. infected
F. annosus	13	0	10	8
L. lundbergii	15	2	10	10
P. gigantea	14	1	10	9
S. sanguinolentum	10	2	10	9

The low frequency of infection of 'double-leader stumps' was associated with a high resin content: infected 'stumps' had a much lower resin content. The results provided further suggestive evidence that copious resin accumulation increases the resistance to fungal invasion. Further discussion of these results is presented later (p. 473).

Seasonal Variation in the Incidence of Natural Infection

Seasonal variation in the incidence of stump infection by *F. annosus* has been discussed by Rishbeth (1951*a*). Certain general trends were observed throughout the years concerned, and he suggested that two of the most important factors affecting the incidence might be (1) the number of spores of this fungus present in the air, and (2) the number of spores of competing fungi present.

Experiments were set up to determine the extent of natural variation in the incidence of stump infection by *F. annosus*, *P. gigantea*, *S. sanguinolentum*, and various species causing blue-stain. Since identification of the last-named was difficult by direct inspection, they were arbitrarily grouped together as 'blue-stain' spp. Previously unthinned forest compartments 18–26 years old were thinned in successive months during 1956 and 1957. Of the stumps produced each month, half were creosoted immediately and the remainder left untreated. Creosoted stumps were expected to act in some measure as controls for aerial infection (Rishbeth, 1951*a*, 1952, 1957). Between 20 and 30 random samples were taken from creosoted and untreated stumps in each monthly plot 3–5 months after felling. The extent of infection was expressed in two ways:

(1) 'frequency' was the percentage of stumps infected (Tables 6(*a*) and 7(*a*)),

(2) 'abundance' was the mean percentage area of stump section colonized for the samples from a given plot (Tables 6(*b*) and 7(*b*)).

TABLE 6

Seasonal Fluctuations in the Incidence of Stump Infection by Various Fungi in a Plantation (H. 17) thinned during Successive Months in 1956

(*a*) *Percentage of stumps infected.*

Stump treatment	Fungus	J	F	M	A	M	J	J	A	S	O	N	D	Average %
None	*F. annosus*	20	14	10	52	67	60	73	55	33	36	75	86	48
	P. gigantea	80	100	90	43	38	50	56	95	100	100	95	95	79
	S. sanguinolentum	25	14	15	0	0	0	14	20	14	23	20	19	14
	'Blue-stain' spp.	80	30	5	33	19	25	45	20	10	27	5	52	29
Creosoted	*F. annosus*	0	0	0	0	4	0	0	0	10	0	4	12	3
	P. gigantea	5	11	4	14	12	5	0	9	15	8	4	10	8
	S. sanguinolentum	0	0	5	0	0	0	0	0	0	0	10	0	1
	'Blue-stain' spp.	15	0	0	14	4	5	0	5	0	0	0	10	4

(*b*) *Estimates of abundance of* F. annosus *and* P. gigantea *in untreated stumps.*

Fungus	J	F	M	A	M	J	J	A	S	O	N	D	Average %
F. annosus	4	1	1	32	36	49	40	24	4	9	11	15	19
P. gigantea	68	82	78	43	31	29	33	72	86	73	81	68	62

The abundance of *S. sanguinolentum* and 'blue-stain' spp. is not recorded here, since the values were generally less than one per cent. Also, for economy of time, sampling of creosoted stumps was discontinued in 1957.

The data for the 1956 experiment show that application of creosote considerably reduced the incidence of stump infection, suggesting that the majority of infections in untreated stumps had proceeded from the exposed stump surface. Breakdown of control was often associated with extensive damage to the stump bark, caused at the time of felling. The creosote layer frequently failed to cover the damaged regions, and it was often obvious that these regions had provided points of entry for infection. Another possible cause of breakdown was the development of fissures in the surface of some stumps, after applying creosote. Since none of the experimental plots contained old stumps, the possibility of trees being infected by *F. annosus* at the time of felling was very slight; as Rishbeth (1951*b*) has demonstrated, this fungus normally infects living trees through root contacts with infected stump roots.

The species occurring most often in untreated stumps were *F. annosus*, *P. gigantea*, and 'blue-stain' spp., while *S. sanguinolentum*, although occasionally common, was generally less frequent. Variation in frequency and abundance between different compartments was most marked for *F. annosus*. It is seen that this fungus occurred most often in stumps at H. 22, and least frequently in those at S. 53.

TABLE 7

Seasonal Fluctuations in the Incidence of Stump Infection by Various fungi in Six Plantations thinned during Successive Months in 1957

(*a*) *Percentage of stumps infected.*

Plantation No.	Fungus	J	F	M	A	M	J	J	A	S	O	Average %
H. 22	*F. annosus*	25	41	5	67	96	64	76	81	48	42	54
	P. gigantea	100	100	90	58	56	36	62	95	87	90	77
	S. sanguinolentum	45	27	40	33	12	14	0	5	10	21	21
	'Blue-stain' spp.	35	41	35	33	32	9	29	38	22	16	29
R. 186	*F. annosus*	4	0	8	29	33	24	35	81	64	70	35
	P. gigantea	95	92	100	43	39	52	95	90	86	87	78
	S. sanguinolentum	9	0	12	52	13	0	0	5	18	26	13
	'Blue-stain' spp.	55	20	48	43	30	9	30	10	4	22	27
S. 53	*F. annosus*	20	5	0	29	23	30	15	26	..	..	18
	P. gigantea	85	90	96	43	27	30	35	57	..	..	58
	S. sanguinolentum	15	10	9	10	0	0	0	17	..	..	8
	'Blue-stain' spp.	10	40	25	33	23	35	5	22	..	..	24
Average % for month	*F. annosus*	16	15	4	41	51	39	42	63	61	56	
	P. gigantea	93	94	95	48	41	39	64	81	86	88	
	S. sanguinolentum	23	12	20	32	8	5	0	9	14	23	
	'Blue-stain' spp.	33	34	36	36	28	18	21	23	13	19	

(*b*) *Estimates of abundance of* F. annosus *and* P. gigantea.

Plantation No.	Fungus	J	F	M	A	M	J	J	A	S	O	Average %
H. 22	*F. annosus*	10	12	<1	48	49	10	25	17	6	6	18
	P. gigantea	88	78	72	32	11	14	24	59	61	77	52
R. 186	*F. annosus*	<1	0	<1	15	14	22	15	27	4	7	11
	P. gigantea	92	95	73	21	22	25	59	30	63	83	56
S. 53	*F. annosus*	5	<1	0	9	13	7	3	6	..	..	5
	P. gigantea	67	74	71	17	6	6	16	26	..	..	28
Average abundance for month	*F. annosus*	5	4	<1	24	25	13	14	17	5	6	
	P. gigantea	82	82	72	23	13	15	33	38	62	80	

During both years, several trends in the incidence of stump infection were apparent. Firstly, stump infection by *F. annosus* in 1956 was relatively low from January to March, increasing to a higher level during the spring and early summer months; after a slight decrease in the autumn, frequency of infection again rose to a high value. Similar trends occurred in 1957, but the magnitude of the changes varied from one compartment to another. In both years, abundance followed similar trends until autumn, and then, instead of increasing as did frequency, remained at a relatively low level.

Seasonal trends in frequency of stump infection by *P. gigantea* displayed a sharp contrast with those of *F. annosus*. During the winter and early spring of both years, frequency of infection was high, falling off during late spring and early summer. Later in the year, frequency increased and reached a high value in the winter: abundance followed similar trends. Stump infection by *S. sanguinolentum* followed trends similar to those of *P. gigantea*, although the former was far less abundant. There was little indication of any significant seasonal fluctuation in the incidence of infection by 'blue-stain' spp.

Factors Influencing Seasonal Variation

(*a*) *Spore content of air.* The numbers of viable spores of stump-colonizing fungi present in the atmosphere at the time of felling, and for an interval afterwards, is probably an important factor influencing the extent of stump infection. Preliminary observations on the air spora of forest compartments were made by exposing malt agar plates, but owing to heavy bacterial contamination and overgrowth of the plates by fungi other than stump colonizers, this method was abandoned. Later observations were carried out by exposing sections of wood, which favoured the development of many stump-colonizing fungi while suppressing that of most moulds. Freshly collected lengths of healthy pine stem, about 12 cm. in diameter, were surface-sterilized with methylated spirit and cut into sections 1–2 cm. thick using a flamed saw. Each section was wrapped immediately in oven-sterilized newspaper and then moistened with tap water. At the experimental compartments, some of the sections were unwrapped and placed on metal supports, while control sections were left wrapped. The exposed sections were rewrapped after 40 minutes, all sections were incubated for 10–14 days at 22·5° C. and the number of fungal colonies which developed was recorded. Results obtained from exposures at different dates are given in Table 8.

TABLE 8

Fungi developing on Wood Sections exposed in Two Plantations at Intervals during 1957 and 1958

Compartment No.	Fungus	Mean No. of colonies per section				
		1957				1958
		Feb.	Apr.	May	Sept.	Jan.
H. 22	*F. annosus*	2·2	0·8	1·6	1·2	1·0
	P. gigantea	10·2	1·2	0	9·4	10·8
	S. sanguinolentum	0·2	0	0	0·4	0
	'Blue-stain' spp.	0·4	0	0·6	0·2	0·2
S. 53	*F. annosus*	0·6	0	0·2	0·8	0·6
	P. gigantea	7·6	2·6	0	9·6	11·2
	S. sanguinolentum	0·2	0·2	0	0	0·2
	'Blue-stain' spp.	1·0	0·4	0·2	0·2	0·4

Control sections remained free from infection by basidiomycetes. The most significant feature of these results was the absence of *P. gigantea* on sections exposed during May 1957, suggesting that fewer spores of this fungus were present in the air at this time than during the autumn and winter months. This apparent decrease in number of available spores may account for the decreased incidence of stump infection by *P. gigantea* in stumps produced during late spring and early summer of 1957. Rishbeth (1957) obtained evidence that spores of this fungus were less frequent in the unusually dry August of 1955 than in March and October of the same year.

It is not possible to offer an explanation for seasonal variation in the incidence of stump infection by *F. annosus* by reference to the exposure data alone. However, spores of this fungus were less abundant in S. 53 than in H. 22, possibly explaining the higher incidence of stump infection in the latter compartment.

Spores of *S. sanguinolentum* and 'blue-stain' spp. were less abundant than those of the two other species, and too few spores were trapped to establish any significant seasonal fluctuations.

Surveys were made during 1956 and 1957 of the incidence of sporophores of several stump-colonizing fungi in the vicinity of the monthly-thinned plots. As noted by earlier investigators (Bjørnekær, 1938; Rishbeth, 1951*a*; Parker-Rhodes, 1957), sporophores of *F. annosus* were produced at all seasons. On the other hand, sporophores of *P. gigantea* and *S. sanguinolentum* appeared seasonally, being absent in the spring and early summer months of both years, but abundant on old stumps or forest litter during the autumn and winter months. The absence of sporophores of *P. gigantea* may account for the low numbers of spores detected during April and May 1957, and hence for the decrease in frequency with which it infected stumps. A similar situation may exist for *S. sanguinolentum*.

The effect of temperature on spore discharge was investigated in the laboratory, using freshly collected sporophores of *F. annosus*, *P. gigantea* and *S. sanguinolentum*. It was found that all species discharged basidiospores at temperatures ranging from 0°–22·5° C., but ceased to do so below 0° C., when they were frozen. Sporophores were also maintained at −5° C. for 10 days and then allowed to thaw out: after a few hours, spore discharge was resumed in each case. It is probable that a similar relationship between temperature and rate of spore discharge exists in the plantations (Bjørnekær, 1938; Rishbeth, 1951*a*, 1957).

Further observations indicated that *F. annosus* is more drought-resistant than the two other species. Sporophores of *P. gigantea* and *S. sanguinolentum* dried out during the dry spring of 1956 and 1957, becoming horny and parchment-like and ceasing to discharge spores. The wet summers of both years were accompanied by a rapid reappearance of actively discharging sporophores. On the other hand, sporophores of *F. annosus* continued to discharge spores at all seasons, being little affected during dry conditions.

The position of spore sources relative to stumps may influence the local incidence of stump infection. Gregory (1945, 1952) has shown that the theory of eddy diffusion, formulated by Sutton (1932), can be applied to problems concerning the dispersal of air-borne spores. Observed gradients of infection by air-borne spores originating from a point source were closely predicted by this theory. In the present investigation, the choice of the monthly-thinned compartments was based partly on their positions relative to known sources of *F. annosus* sporophores. It was hoped that some information might be obtained about the effect of distance of spore source on the frequency of stump infection. *F. annosus* was the fungus most likely to give any indication of such effects, since no sporophores were present in the experimental compartments, whereas those of *P. gigantea* and *S. sanguinolentum* were ubiquitous on fallen branches and other litter. Reference to Table 6(*a*) and 7(*a*) shows that *F. annosus* occurred far less frequently in compartment S. 53 than in H. 17, H. 22, or R. 186. This finding was surprising, considering that there was an extensive source of sporophores about 250 yards due east, separated from S. 53 only by a strip of grassland. A possible explanation of these results is that the prevailing winds during the 2 weeks following each felling were generally towards the spore source. The other compartments were more or less surrounded by sporophore sources, and it is probable that changes in wind direction did not influence the incidence of stump infection to such an extent as in S. 53. These tentative conclusions agree with Rishbeth's (1951*a*) suggestion that local variation in the abundance of *F. annosus* sporophores will be reflected in the incidence of infection.

An experiment was set up in 1956 to determine whether the introduction of sporophores of *F. annosus* into a compartment would affect the incidence of stump infection. The compartment was situated on the western edge of the main forest area and was relatively isolated from any immediate sporophore sources. A total of 24 stumps bearing large sporophores, 12 in January and a further 12 in June, were extracted from another compartment and 'replanted' so that the sporophores were just above ground level: mosses and needle-litter were placed around each sporophore to retard drying-out. The production of spores was tested immediately, and then at intervals during 1956, by exposing malt agar plates, facing into the wind close to the sporophores. Between 20 and 30 trees were felled at the end of each month, and the stumps were sampled after 3–5 months in order to record the extent of infection. The results are presented in Table 9.

TABLE 9

The Effect of Spore Production on the Incidence of Stump Infection by F. annosus *in a Plantation thinned during Successive Months in 1956*

	J	F	M	A	M	J	J	A	S	O	N	D
Frequency of infection (%)	10	4	5	9	4	0	25	29	75	85	43	33
Estimates of abundance (%)	3	<1	<1	2	<1	0	12	4	11	9	7	5
No. of sporophores giving spore deposits	10	2	0	1	0	2	16	14	14	8	12	4
			Out of 12						Out of 24			

Although no means were apparent for providing a suitable control, it is highly suggestive that an increase in spore production later in the year was accompanied by an increase in the frequency of infection.

The distribution of stumps containing *F. annosus*, relative to the sporophores, was investigated. However, there were no indications of infection gradients. This was not surprising, since the distances between neighbouring sporophores were relatively short, and it is likely that any gradients would overlap to produce a more or less uniform distribution of infection.

(*b*) *Competition between fungi.* Although seasonal variation in the incidence of stump infection by *P. gigantea* could be related to rate of spore discharge, this was not always possible for *F. annosus.* Observations and experiments, to be described elsewhere, have shown that intense competition for successful colonization exists in stumps immediately after initial infection. *P. gigantea* is the most vigorous competitor, often replacing other fungi in stumps. A factor influencing the chance of successful colonization by *F. annosus* is the proportion of spores, relative to that of *P. gigantea* spores, which initially infect the stump. For successful colonization by *F. annosus*, the ratio—number of *F. annosus* spores: number of *P. gigantea* spores—must be high. It is now possible to offer an explanation for the low incidence of stump infection by *F. annosus* during the autumn and winter of 1956 and 1957. *P. gigantea* restricted stump colonization by *F. annosus* owing to the balance of spores being in its favour. During the spring and early summer months, the decrease in number of *P. gigantea* spores resulted in the pressure of competition being reduced, enabling *F. annosus* to colonize stumps to a greater extent. The striking inverse relationship between the abundance data for the two fungi lends support to this explanation.

(*c*) *Other factors.* There was no indication of any seasonal fluctuation in the amount of resin accumulating in stumps after felling. On the other hand, the moisture content of stump samples taken during spring and early summer months was higher than that of those removed at other times. Further research on the moisture requirements of stump-colonizing fungi is necessary in order to assess the possible effects on colonization.

ALTERNATIVE METHODS OF STUMP INFECTION

Infection Prior to Felling

Trees which had been killed by *F. annosus* were felled and samples taken from the stumps. It was found frequently that the fungus was widespread throughout the roots and body of such stumps. Stumps of trees which had been dead for a considerable time were often also colonized by saprophytes such as *P. abietinus* and *S. sanguinolentum.*

Trees killed by suppression were commonly encountered in unthinned plantations. A survey of the frequency of fungi in 50 stumps of these trees immediately after felling showed that 41 contained various blue-stain fungi, 31 *P. abietinus* and 28 *S. sanguinolentum*: samples from 8 stumps were sterile.

These findings emphasize the importance of selecting stumps of known history when setting up experiments.

Invasion of Stump Roots by Saprophytes

(*a*) *Natural infection.* Observations were made on stumps of different ages in order to assess the relative importance of this possible mode of infection.

Lateral roots were excavated and severed close to the body of the stump. In addition, a large portion of the stump body was removed by severing at the level of the uppermost lateral roots. The roots were washed, split longitudinally and incubated in the manner described previously: stump sections were also split and incubated. Discoloration of infected wood provided a useful guide for delimiting the extent of colonization by decay fungi. The distribution of basidiomycetes other than *F. annosus*, *P. gigantea*, and *S. sanguinolentum* is shown in Table 10.

TABLE 10

The Distribution in Stumps of Various Ages of Basidiomycetes Other than those which had colonized the Cut Surface

		Percentage of stumps infected		
		Body of stump	Stump roots*	
Age of stumps	Total No. of stumps		Proximal 1 ft.	Distal portion
6 months	25	0	0	0
1 year	45	0	2	4
2 years	40	3	3	10
3 years	30	73	77	90
4 years	26	51	76	100

* Percentage based on the number of stumps which had one or more roots infected.

The distribution of *F. annosus*, *P. gigantea*, and *S. sanguinolentum* in stumps up to 1 year old provided conclusive evidence that they had infected stumps through the cut surface. However, other basidiomycetes frequently occurred in 3–4-year-old stump roots, and it was apparent that invasion by these fungi was proceeding from the roots, or buried portions of the stump body, towards the cut surface. *H. fasciculare* was the most frequent invader of 2–3-year-old stump roots: mycelial sheets and strands were abundant between the bark and wood. In older stumps, this fungus was frequently widely distributed throughout the stump body. *T. rutilans* was found only in stump roots older than 3 years. During its early stages of invasion this fungus was often restricted to the more distal regions of the root: more extensive colonization was evident in older stumps.

Many other fungi probably invade stump roots in a similar manner, possible examples being *P. abietinus* and *Paxillus atrotomentosus*.

(*b*) *Experimental infection.* An experiment was set up in a previously unthinned plantation of 26-year-old Scots pine. A total of 40 trees were felled, and the stumps were creosoted immediately to reduce the incidence of infection at the cut surface. One lateral root of each stump was carefully exposed for about 2 feet from the stump body, and then inoculated with a pure culture of one of the test fungi. Inoculum was prepared as follows. Small plugs, 1 cm. in diameter and 5–8 mm. long, were cut out of pine wood sections with a cork-borer. After autoclaving, the plugs were transferred to pure cultures of the required fungi growing on malt agar. They were incubated at 22·5° C.

for 3 weeks, by which time they were thoroughly permeated by the fungi. Inoculations were performed by taking a plug of wood from each root, after washing the root surface with methylated spirit, and inserting the inoculum plug in its place. Control roots were inoculated with sterile plugs.

The wounds were sealed with grafting wax and roots were re-covered with soil. The inoculations were examined 15 months later and the extent of colonization was determined in each case. The results are given in Table 11.

TABLE 11

Results obtained by Inoculating Stump Roots with Woody Inocula of Various Fungi

Fungus	No. of roots infected (out of 5)	Mean extent of growth towards stump (cm.)
Ceratocystis minor	5	42*
Fomes annosus	5	30*
Hypholoma fasciculare	3	3
Peniophora gigantea	5	38*
Polystictus abietinus	0	0
Stereum sanguinolentum	4	22
Tricholoma rutilans	1	<1

Control 2 roots infected by *P. gigantea*.

* Denotes that the fungus had grown into the body of the stump.

The ability of *H. fasciculare*, *P. abietinus*, and *T. rutilans* to colonize fresh stump roots was low compared with that of the other fungi. *H. fasciculare* had caused appreciable decay immediately around the point of inoculation but was not present in wood beyond this region: however, mycelial strands frequently extended beyond the decayed regions, between the bark and wood. *F. annosus*, *P. gigantea*, and *C. minor* had in some instances entered the body of the stump, and were invading in the direction of the cut surface. The presence of *P. gigantea* in two control roots was explained by the occurrence of aerial infection of the stump surface.

Splash-infection

It is likely that spores of stump-inhabiting fungi are dispersed not only by air currents but through the agency of rain-splash. Large numbers of spores, including those of stump fungi, have been detected on crowns of pines and in water droplets falling from the tree canopy just after rain (Rishbeth and Meredith, 1957). Such droplets might well infect stumps under some circumstances. An experiment was designed to estimate the relative importance of air currents and rain-splash in stump infection. Three sets each of 20 stumps were prepared: in the first, stumps were covered by a bell-shaped splash deflector, constructed of wire and moisture-proof 'Cellophane', allowing considerable air circulation around the stump. Indicators showed that no water drops reached the stump surface, despite the occurrence of several heavy showers. In the other two sets stumps were normally exposed, but in one they were inoculated with washings of pine foliage. Results of two experiments, in which the abundance of *F. annosus* and *P. gigantea* was determined by sampling after 6 weeks, are recorded in Table 12.

TABLE 12

Effect of excluding Rain-splash from Stumps on the Incidence of Infection by F. annosus *and* P. gigantea: *Abundance of Infection in Two Experiments*

Sampling date	Fungus	Stump treatment: Covered	Uncovered	Uncovered, Inoculated
25th June 1957	*F. annosus*	23	44	52
	P. gigantea	10	26	23
14th Oct. 1957	*F. annosus*	16	23	32
	P. gigantea	28	41	49

Both species were present in the needle-washings used for the experiments, which accounts for the somewhat greater stump infection in the inoculated sets. Although in both experiments *F. annosus* and *P. gigantea* were rather more abundant in exposed stumps than in covered ones, only a small proportion of the infections can be ascribed to splash-borne spores, assuming covered stumps to have been infected by air-borne spores only. Under other conditions, perhaps especially where the tree canopy is denser or the rainfall heavier, splash-infection may assume greater relative importance.

To discover whether this potential source of stump infection varies in magnitude throughout the year, estimates were made at intervals of the number of *F. annosus* and *P. gigantea* spores on pine foliage in three plantations where monthly thinning was in progress. Approximate counts per g. of needle sample, obtained by plating suspensions on freshly cut pine sections, are given in Table 13. *P. gigantea* spores were notably scarce on foliage samples collected in April, May, and June. Counts of *F. annosus* spores showed some seasonal variation and also differed with the locality, being lowest in plantation S. 53. These findings agree with results obtained by exposing wood sections at the same sites.

TABLE 13

Approximate Estimates of the Numbers of Spores of F. annosus *and* P. gigantea *per gram of Pine Needle Sample*

Fungus	Plantation No.	Month of sampling (1957): Jan.	Apr.	May	June	Aug.	Oct.	Dec.
F. annosus	H. 22	80	40	5	240	120	160	48
	R. 186	40	8	28	320	280	40	200
	S. 53	44	11	5	20	12	20	12
P. gigantea	H. 22	120	8	1	1	80	320	200
	R. 186	160	8	4	4	80	160	320
	S. 53	52	10	1	0·5	1	20	44

DISCUSSION

The present studies on stump-inhabiting fungi have shown that relatively few normally colonize freshly exposed stump surfaces. The colonizing species are chiefly basidiomycetes and ascomycetes, the latter being represented by several species of *Ceratocystis*. Although many of these are not restricted to pine stumps, for instance *S. sanguinolentum*, which frequently colonizes dead trees and fallen branches, they form a fairly well defined ecological group of stump-inhabiting fungi. It is proposed to refer to them here as 'stump-surface colonizers'. For various reasons, as yet little understood, freshly cut stumps appear to be selective for such fungi. The inclusion of *F. annosus* in this ecological group confirms the findings of Rishbeth (1951*a*), while the inclusion of several other species suggests that this mode of stump infection is more widespread than has previously been realized. The investigations of Käärik and Rennerfelt (1957) on the fungal flora of spruce and pine stumps in Sweden showed that 'stump fungi' occurred more frequently in samples taken from the top of stumps than in those from the roots. They interpreted this as indicating that most of the infection was initiated by air-borne spores falling on the stump surface; however, no experimental evidence was presented to support this interpretation.

A high resin content of stumps appears to increase resistance to infection. Failure to infect 'stumps' of 'double-leaders' by experimental inoculation agrees with the observation that similar 'stumps' rarely become naturally infected. Although high resin content is a significant feature of these 'stumps', other physiological factors may increase resistance to a level higher than that in normal stumps, where there is no organic connexion with a healthy tree. The freshly exposed surface of a 'stump' of a 'double-leader' may be compared with a wound in the stem of a living tree. Rishbeth (1951*b*) compared resistance to infection of suppressed and vigorous trees after inoculation of stem wounds with *F. annosus* basidiospores: infection occurred only in trees greatly weakened by suppression. Similarly, in the present investigation it was found that only 'stumps' of suppressed 'double-leaders' became infected after inoculation with various fungi.

Seasonal variation in the incidence of stump infection is related to several factors. The results obtained by exposing wood sections in the plantations indicated that the number of spores of *P. gigantea* decreased during relatively warm, dry periods. Data derived from needle samples taken from the crowns of pines at intervals during 1957 also suggested that the spore population underwent similar fluctuations. This apparent decrease in number of available spores may account, in part, for the corresponding decrease in the extent of stump infection by *P. gigantea* during spring and early summer months. Although sporophores of *F. annosus* were produced at all seasons, there was an increase in the frequency of stump infection by this fungus during spring and early summer. The striking inverse relationship between frequency and abundance data for *F. annosus* and *P. gigantea* suggests that the former species was excluded from many stumps during autumn and winter months by competition from *P. gigantea*. When the pressure of competition decreased, *F. annosus* often became dominant.

Seasonal trends in the incidence of stump infection by *F. annosus* contrast with those observed by Rishbeth (1951*a*), who found that there was a tendency for stump infection to be slight in May and June and to increase in late summer or autumn. The difference between these observations may be due to an increase in the abundance of *P. gigantea* in the plantations over the last ten years (Rishbeth, 1958), so that now *F. annosus* is often subject to greater competition.

There is often a considerable delay between the time of felling and the invasion of stump roots by fungi present in the soil or litter-layers. It is possible that several stump-colonizing fungi are present in the rhizosphere (Hiltner, 1904) of a pine tree root-system. However, the peridermal tissues of the root provide a mechanical resistance to fungal invasion, and breakdown of this barrier is a relatively slow process. Experimental inoculations showed that even when this barrier was removed, certain fungi, for instance *H. fasciculare* and *T. rutilans*, made relatively slow growth in fresh roots. This may explain why these fungi do not invade stump roots extensively until several years after felling. Thus stump-surface colonizers have an initial advantage in colonization. This conclusion was confirmed by studies on the progress of colonization, which will be described elsewhere.

SUMMARY

The ecology of *Fomes annosus* and other stump-inhabiting fungi has been studied in East Anglian pine plantations, where *Pinus sylvestris* and *P. nigra* var. *calabrica* are the commonest species. Methods of sampling stumps are described and a list of fungi found on pine stumps is given.

Several fungi infect the stump surface very soon after felling: *F. annosus*, *Peniophora gigantea*, *Stereum sanguinolentum*, and several blue-stain fungi are the most frequent 'stump-surface colonizers'. The incidence of natural infection is somewhat reduced by covering stumps with 'Cellophane' sheets, or more effectively, by applying creosote to the cut surface immediately after felling. After inoculating the freshly exposed surface with spore suspensions, stumps were colonized by some fungi but not by others. There was no indication that stumps of dominant trees differ greatly in susceptibility to infection from those of suppressed trees. There is some evidence that high resin content of stumps increases resistance to infection.

In 1956 and 1957, *P. gigantea* was most frequent in stumps produced during autumn and winter and least frequent in those produced during spring and early summer. The extent of stump infection by *F. annosus* was inversely related to that of *P. gigantea*. The frequency of *S. sanguinolentum* followed trends similar to those of *P. gigantea*. Variation in the spore content of the air is probably an important factor influencing the incidence of stump infection. The varied incidence of infection by *F. annosus* in individual plantations

was related to the spore counts obtained by exposing wood sections. It is probable that competition from *P. gigantea* is partly responsible for the relatively low abundance of *F. annosus* in stumps produced during autumn or winter.

Stump roots are invaded by saprophytic fungi present in the soil or litter layers, although this does not usually occur for several months after felling. Inoculation of fresh stump roots showed that certain fungi rapidly colonized them, whereas others grew slowly or not at all.

Although spores of several stump-colonizers can be detected in water drops falling from the tree canopy, the results of two experiments suggested that the proportion of stump infection ascribable to water-borne spores was low in comparison with that caused by wind-borne spores.

During 1957, the population of *P. gigantea* spores on pine foliage was lowest in spring and early summer. The abundance of *F. annosus* spores on foliage varied with the locality.

ACKNOWLEDGEMENTS

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Geminitheca scotica gen. et sp. nov.: a Pteridosperm from the Lower Carboniferous of Dunbartonshire

BY

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With two Plates and five Figures in the Text

ABSTRACT

This article gives an account of the morphology of the ovulate cupules and microsporangia of *Geminitheca scotica* gen. et sp. nov., a new type of pteridosperm from the Lower Carboniferous of Dunbartonshire. The cupules are united in pairs and are borne in bunches at the tips of naked dichotomizing rachides. Evidence is put forward to show that the cupules are probably derived from a system of dichotomizing rachides modified to enclose terminal ovules. Each cupule contains two ovules similar in form to those of *Calathospermum scoticum* Walton, but differing in possessing a free integument. The elongated microsporangia also are borne in terminal bunches. The microspores have a cingulum.

INTRODUCTION

THE classic Lower Carboniferous exposure near the Loch Humphrey Burn in Dunbartonshire is a very rich source of plant remains and several new species of fossil plants have been described from it: *Lyginorachis trinervis* Calder (1935), *Calathospermum scoticum* Walton (1949*a*), *Alcicornopteris hallei* Walton (1949*b*), *Eristophyton waltoni* Lacey (1953), and *Protopitys scotica* Walton (1957). Associated with these are stems of *Archaeocalamites* and numerous leaves of species of *Rhacopteris*, *Rhodea*, *Spathulopteris*, and *Sphenopteridium.* Among this material have been found several specimens of two small fructifications preserved both as compressions and as petrifactions. One fructification consists of small paired cupules each containing two ovules; the other consists of bunches of paired microsporangia and is undoubtedly the male organ of the same species. These are described in this article and are named *Geminitheca scotica* gen. et sp. nov.

The source of the material is a small lenticular bed of fine sandstone in the series of sedimentary rocks which underlie the Clyde Plateau Lavas and which have been ascribed to the Cementstone Group of the Calciferous Sandstone Series. The sandstone bed probably represents the bed of a small lake into which were washed volcanic ash, sand, and plant fragments.

Four petrological sections of a petrified group of empty cupules were prepared by the late W. N. Croft and for the rest several series of peel sections have been made using the Croft parallel grinder. The compressions were investigated largely by means of nitrocellulose 'pulls', and transfer preparations macerated in Schulze's solution.

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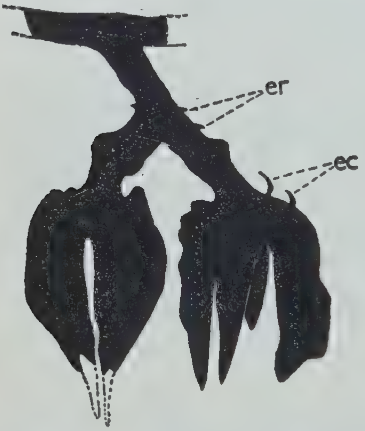
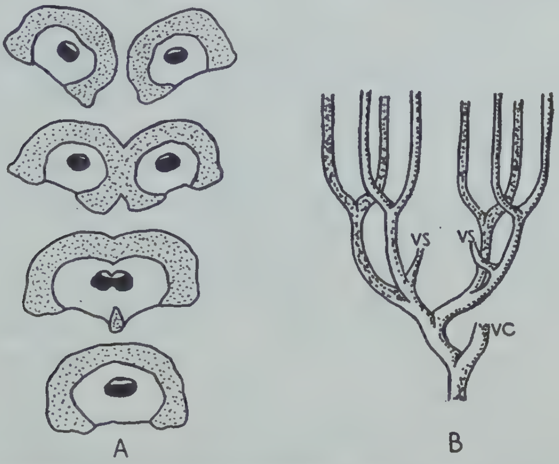
THE RACHIS AND CUPULES

The paired ovulate cupules are united at the base for about a quarter of their length (Text-fig. 1) and are borne in bunches at the tips of naked dichotomizing rachides. While several isolated specimens consisting of one or two pairs of cupules have been found both as compressions and as petrifactions, in most of the compressions and in one petrifaction there are three successive dichotomies of the rachis resulting in a bunch of eight pairs of cupules. Usually the larger bunches of cupules are compressed vertically (i.e. the vertical axis of the cupules is foreshortened) and only one relatively complete specimen has been found in which the bunch is compressed laterally (Pl. 1, Fig. 1). This and the fact that the cupules, although of rather massive construction, are carried at the tip of a relatively slender rachis suggests that they were probably borne pendulously on the parent plant. When compressed vertically the cupule-pairs show considerable distortion and it is impossible to determine their original shape (Pl. 1, Fig. 2). When compressed laterally they appear more or less pear-shaped in outline (Text-fig. 1). They vary from 9 to 12 mm. in length and each cupule attains a maximum diameter of 2 to 4 mm. at about a third of the way from the base.



TEXT-FIG. 1. Silhouette drawing of two pairs of ovulate cupules, ×2·5. The pair on the right is damaged. *ec*, emergence on cupular lobe; *er*, emergence on rachis. (Drawn from specimen Pb2674 in the Hunterian Museum Palaeobotanical Collection, Dept. Bot., Univ. Glasgow.)

As stated above, in the most complete petrified specimen there are three successive dichotomies of the rachis; below the first the rachis has a greatest diameter of 2·5 mm. and a shortest of 1·6 mm.; above the ultimate dichotomy it measures 2·0 by 1·2 mm. (Pl. 1, Fig. 4). The cortex of the rachis is divided into two distinct zones: a sclerotic outer cortex composed of elongated cells probably of a fibrous nature and an inner cortex consisting of axially elongated, parenchymatous cells which frequently have dense, dark brown contents. The outer cortical layer is crescent-shaped and does not extend completely round the rachis. The side from which it is absent is slightly concave, possibly as a result of shrinkage of the parenchymatous tissue, though it may be a natural feature as it occurs in all the rachides examined. In the smaller rachides the cells towards the periphery of the inner cortex are thickened slightly and do not have dark contents. In the centre of the rachis there is a single collateral vascular strand with three protoxylem groups consisting of small, irregularly spiral tracheids. The protoxylem is orientated towards the gap in the outer cortical tissues. In the smaller rachides the metaxylem tracheids are predominantly of a type showing a transition from scalariform to reticulate thickening. In the larger rachides there is a further transition to tracheids showing from one to five rows of bordered pits with transverse eliptical apertures. This is especially clear in 'pulls' of some of the compressions (Pl. 1, Fig. 5). The remains of a small-celled, thin-walled tissue adjoining the metaxylem presumably represents the phloem.

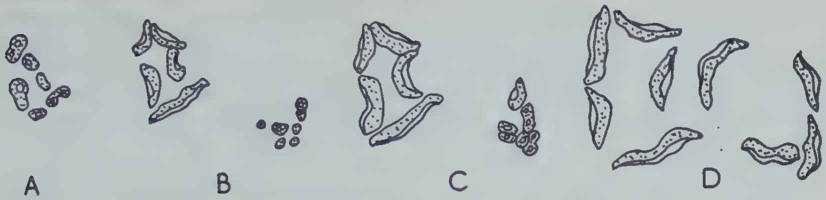


TEXT-FIG. 2. A, Diagrammatic series of sections through a dichotomy of the rachis; B, Diagrammatic reconstruction of the vascular system in the base of a single cupule. *vc*, vascular strand to the other cupule; *vs*, vascular strand to an ovule.

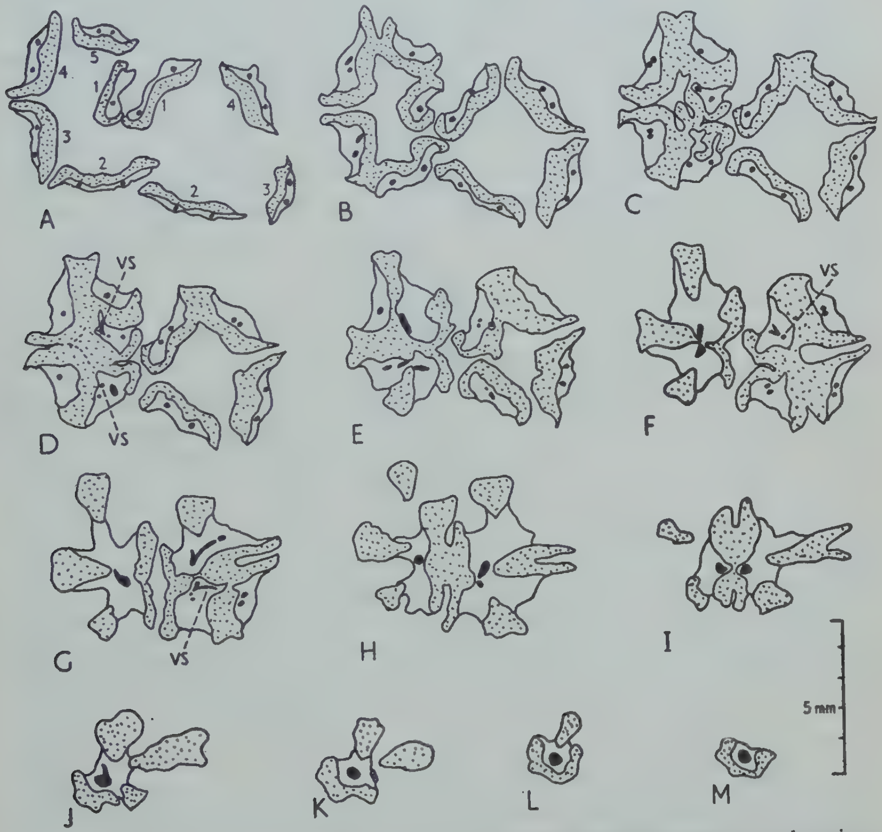
The rachis has a very characteristic mode of branching which is illustrated diagrammatically in Text-fig. 2 A. Below the fork a group of sclerotic cells is developed in the middle of the gap in the sclerotic outer cortex. After the division of the vascular strand a further development of sclerotic tissue between the two daughter strands results in the formation across the centre of the rachis of a complete strip of mechanical tissue. Separation of the two branches is effected by a division along the centre of this tissue. This type of branching, in which forking of the vascular strand is accompanied by the development of sclerotic tissue between the two daughter strands and the subsequent divergence of the branches by cleavage of this tissue, is repeated in all dichotomies of the rachis and in a modified form in the cupules themselves. The branching of the rachis is very regular. The branches diverge at an angle of about 120° and the plane of the dichotomy is at an angle of about 120° to the parent rachis. In the cupules the angles of divergence of the vascular strands are not constant.

Immediately below the base of the cupule-pair four peripheral groups of sclerotic tissue arise successively in the gap in the outer cortical tissues of the rachis (Text-fig. 4 I–L). The outer cortex becomes reduced and divides into two parts, so that at the junction of the cupules and the rachis there are altogether six peripheral groups of sclerotic tissue. When the vascular strand forks in the base of the cupule-pair two of these groups, on opposite sides of the base, are connected by a further development of sclerotic tissue between the two daughter strands. The two cupules become separated by a division down the centre of this tissue (Text-fig. 4 G–I). In many cases the groups of sclerotic tissue form processes which project backwards from the base of the cupule (Text-fig. 1). This feature is shown by all the cupules examined and appears to be a natural phenomenon, not the result of decay or distortion of the tissues. The point at which the groups of sclerotic tissue arise varies considerably in different cupule-pairs. In the pair illustrated one group is not formed until after the division of the vascular strand (Text-fig. 4 I). In the base of each cupule a further group of sclerotic cells arises at the periphery, so that in transverse section near the base the cupule has a central vascular strand surrounded by a tissue of thin-walled cells with dark contents, corresponding to and continuous with the inner cortex of the rachis, and four unequal, peripheral groups of sclerotic tissue (Text-fig. 4 G, H). In the right-hand cupule of the pair illustrated two successive dichotomies of the vascular strand, accompanied by the development of sclerotic tissue and subsequent cleavage, have resulted in the separation first of two lobes and then of four, each with a single vascular strand. In lobes 1 and 2 a further division of the vascular strand occurs; in each case one branch enters the base of an ovule and the other supplies the lobe. Thus in effect the two ovules are borne on cupule lobes (Text-fig. 4 F, G). In the left-hand cupule of the pair illustrated the situation is more complex owing to the presence of a fifth lobe. One branch of the main cupular vascular strand supplies lobes 2 and 3 and the other lobes 4, 5, and 1. The latter strand divides: one branch supplies lobe 4, while the other branch forks, one strand passing into one of the two ovules and the other dividing to supply lobes 5 and 1. The vascular strand of the other ovule arises as a branch of the strand which supplies lobe 2.

At and above the point where the lobes become free each has a layer of sclerotic tissue directed towards the cupular axis and an abaxial layer of parenchymatous tissue identical with the inner cortical tissue of the rachis. The edges of the sclerotic layer are curved outwards (Pl. 1, Fig. 9). In the base of each lobe the vascular strand forks and the two daughter strands pass up the abaxial layer. An exception to this is lobe 1 of the left-hand cupule of the illustrated pair where the strand remains undivided. Since the strands in the lobes divide in the same way as that in the rachis the phloem is orientated towards the axis of the cupule and the xylem towards the outside (Pl. 1, Fig. 6). The xylem of the cupular strands consists of eight to twelve tracheids showing a transition from scalariform to reticulate thickening. A few tracheids with biseriate bordered pitting are occasionally present. The division of the strand in the lobe differs from all other divisions of the vascular strand in that the associated development of sclerotic tissue is delayed. At the apex of most lobes the edges of the adaxial layer become increasingly recurved and at the same time sclerotic tissue develops between the two vascular strands. The final result is that each strand, surrounded by parenchymatous tissue, is completely enclosed by sclerotic tissue (Text-fig. 3 A).



TEXT-FIG. 3. Drawings of a series of sections through the apex of a pair of empty cupules; sclerotic tissue is stippled. (Drawn from slides 584, 585, 586, and 588 in the Figured Slide Collection, Dept. Bot., Univ. Glasgow.)



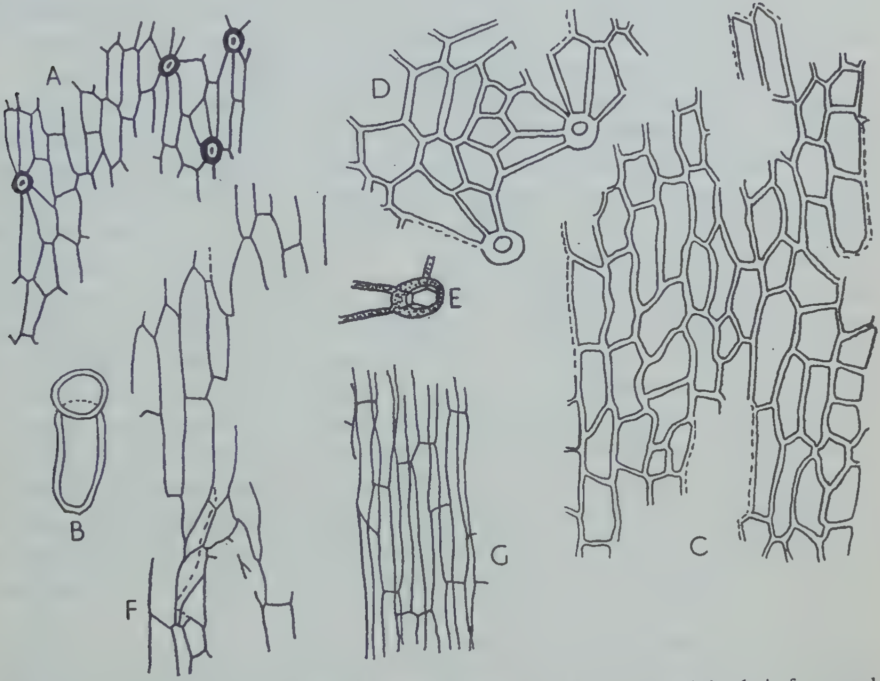
TEXT-FIG. 4. Drawings of a series of sections through the base of the same cupule-pair as in Text-fig. 3; sclerotic tissue stippled; *vs*, vascular strand to an ovule. The lobes are numbered for convenience in description. (Drawn from slides 1182, 1185, 1187, 1188, 1190, 1192, 1194, 1197, 1200, 1204, 1208, and 1210 in the Figured Slide Collection, Dept. Bot., Univ. Glasgow.)

Division then occurs along the centre of the sclerotic tissue between the strands, thus completing the dichotomy. In a few lobes there is no development of sclerotic tissue between the strands and the dichotomy is never completed. The tips of the lobes are composed entirely of sclerotic tissue. The vascular system fn the base of a single four-lobed cupule is illustrated diagrammatically in Text-fig. 2 B.

In the compressions small fragments of the epidermis of the rachis and cupules are preserved but as they are partly petrified they disintegrate in acids and therefore the cuticles cannot be isolated by maceration. However, reasonably satisfactory observations have been made by means of nitrocellulose 'pulls' of small areas. All the epidermal structures figured are from the specimen illustrated in Pl. 1, Fig. 1. The epidermal cells of the rachis are axially elongated with thin, moderately straight walls, and are interspersed with numerous hair bases, each consisting of a small, rounded, thick-walled cell (Text-fig. 5 A). Each hair base is surrounded by six to eight elongated polygonal cells radiating out from it in a characteristic manner. The hair, which frequently is still present either compressed vertically on to the base or lying detached nearby, consists of an elongated stalk cell bearing a rounded apical cell (Text-fig. 5 B). The stomata, which are orientated longitudinally, are very sparsely scattered and of the few found only one is fairly complete (Pl. 2, Fig. 10). In it lateral subsidiary cells have been cut off from the neighbouring epidermal cells but there is no sign of any anticlinal division in the polar cells. The outline of the sunken guard cells is discernible below the subsidiary cells.

The inner epidermis of the cupule consists of irregular, more or less rectangular cells which tend to be arranged in short vertical rows, possibly preserving the outline of the original meristematic cells (Text-fig. 5 C). The cell walls are fairly uniformly thickened. In some cupules the cuticle and cell walls are not preserved but the outline of the cells is demarcated by the dense, dark brown cell contents. The outer epidermis, which is composed of irregular polygonal cells, has numerous hair bases with somewhat elongated polygonal cells radiating out from them (Text-fig. 5 D). In some compressions these hair bases and their associated cells are the only epidermal features preserved. Usually part of the hair is compressed on to the base obscuring its structure, but in some cases where the hair appears to have been broken off before fossilization the base is visible as a small, thin-walled, polygonal cell (Text-fig. 5 E). The hairs are of two types: long, multicellular, uniseriate hairs, which are quite numerous, especially between the cupules (Pl. 1, Fig. 8); and short, bicellular, capitate hairs like those on the rachis but so far only found in petrifactions. It has proved impossible to determine the nature of the base of the capitate hairs since all the bases examined in compressions appear to belong to the multicellular hairs.

Two types of emergence are present (Text-fig. 1), one on the outside of the cupular lobes (Pl. 1, Fig. 9) and one on the side of the rachis where the sclerotic outer cortex is absent (Pl. 1, Fig. 7). Those on the cupular lobes are usually about 2 mm. long, 0·1 mm. in diameter for most of their length but broadening rapidly at the base to 0·5 mm. or more, and composed throughout of small, elongated, thick-walled cells, indicating that they were quite stiff, rigid structures. Those on the rachis are 0·5 to 0·8 mm. long, 0·5 mm. broad at the base and curved upwards at the tip. The base, which is covered by a continuation of the epidermis of the rachis with several hair bases, is parenchymatous but there is a rapid gradation towards the apex where the cells are small, thick-walled, and elongated, resembling those of the cupular emergences. Most of the emergences on the rachis have the appearance of being broken at the tip, suggesting that initially they may have been like those on the cupules.



Text-fig. 5. A, epidermis of rachis of ovulate cupules, × 130; B, bicellular hair from rachis of ovulate cupules, × 260; C, inner epidermis of cupular lobe, × 130; D, outer epidermis of cupular lobe, × 130; E, hair base from outer epidermis of cupular lobe, showing thin-walled, polygonal basal cell, × 260; F, outer epidermis of integument, × 130; G, fused inner cuticle of integument and nucellar cuticle, × 130. (All epidermal structures are drawn from nitrocellulose 'pulls' of specimen Pb2711 (Pl. 1, Fig. 1) in the Hunterian Museum Palaeobotanical Collection, Dept. Bot., Univ. Glasgow.)

The Ovules

So far only two pairs of petrified cupules have been found which still contain ovules. In both cases, as preservation is rather poor, very little detailed structure is visible, especially at the base of the ovules. The outstanding feature of the ovules is a cupule-like integument which on the evidence stated below is free from the nucellus except at the extreme base. Apart from this feature they resemble the ovules of *Salpingostoma dasu* Gordon (1941) and *Calathospermum scoticum* Walton (1949*a*).

The ovule is borne on a very short pedicel and has usually four but in one case three prominent longitudinal ridges. The integument is divided into usually eight lobes which become free at about the level of the base of the lagenostome. In some ovules the number of integumentary lobes is not clear owing to poor preservation, but it appears to vary from six to nine with eight as the commonest number. The integument is composed of a uniform tissue of thin-walled, much elongated cells. Each lobe possesses a single vascular strand but it is impossible in the present material to determine the orientation. The xylem of the strand consists of two or three small scalariform tracheids. There is no vascular tissue in the nucellus, which is represented merely by a thin layer of small, badly preserved cells with dark contents. The megaspore membrane is well preserved in compressions as well as in petrifactions.

In compressions all that is usually preserved of the outer epidermis of the integument is the dark cell contents and only one small fragment showing cell walls has been found (Text-fig. 5 F). The cells are thin-walled, long and narrow. At one end of the fragment the cells are rather irregularly shaped and much smaller in size, appearing as if several anticlinal divisions have occurred, as a result possibly of a wound or the presence of an emergence. The inner cuticle of the integument and the nucellar cuticle are inseparably fused together to form a double membrane on which the outlines of both sets of epidermal cells are visible. The presence of these two fused cuticles is taken as clear evidence that the integument and nucellus are free. According to Harris (1926) where the integument and nucellus are continuous there is no cutinized membrane between the outer cuticle of the integument and the megaspore membrane. As the fused cuticles will not withstand maceration and as they are covered by a thick layer of coaly matter it has proved impossible to isolate them. The largest fragments were obtained in nitrocellulose 'pulls'. In both cuticles the cells are extremely long and narrow but because few transverse walls are present in the fragments examined (Text-fig. 5 G) the outline of a complete cell has not been found.

With the megaspore membrane maceration has been successful and one complete one and several fragments have been isolated from transfers of cupule-pairs. The complete membrane obtained (Pl. 2, Fig. 12) is 4·9 mm. long and has a maximum diameter of 1·2 mm. Microtome sections of wax-embedded fragments reveal that it is composed of two distinct layers: an inner homogeneous layer and an outer granular layer, each about 3μ in thickness at the middle of the membrane. In several specimens, including that illustrated, irregularly distributed perforations are present. That they are not a constant feature suggests that they are of secondary origin, probably as a result of decay before fossilization. In one petrified ovule, inside the membrane and just below its apex, there is a small mass of tissue showing very little cell structure, which must be the remains of the prothallus. Within it are two rounded conical bodies which show no cell structure and which appear to be archegonial eggs (Pl. 2, Fig. 11).

The ovule has a large lagenostome with a dome-shaped roof composed of small, elongated cells with dark contents, and prolonged at the apex into a tubular salpynx (Pl. 2, Fig. 11) which extends for at least 2 mm. The megaspore membrane, forming the floor of the lagenostome, projects for some distance into the cavity. The free lobes of the integument extend upwards as long slender processes surrounding the salpynx and at least equalling it in length. Microspores are present in the lagenostome and salpynx of all the petrified ovules found, as many as twenty-two being present in one case. Of these, twenty-one are all of the same type and spores of this type are the only ones found in other ovules (Pl. 2, Fig. 13). Similar spores have been isolated from the small microsporangiate fructifications described below.

THE MICROSPORANGIA

Only two compressions of the microsporangiate fructification have so far been found but there is evidence that a small bunch of petrified microsporangia from the same locality, represented by three peel sections in the Figured Slide Collection of the Botany Department, University of Glasgow, is of the same species. The tassel-like bunches of sporangia (Pl. 2, Fig. 13) bear a superficial resemblance to the fructifications of *Telangium* Benson (1904) and it is quite likely that they were included in the *T. affine* recorded by Kidston (1924) from the 'escarpment on south side of Loch Humphrey Burn'. They differ from *Telangium* in the smaller size of the sporangia which are not united at the base to form a disk of sterile tissue. Although on the whole preservation of the compressions is rather poor a certain amount of detailed structure is visible, particularly in the rachis which is partially petrified. The epidermis of the rachis, with its numerous hair bases and sparsely scattered stomata, is indistinguishable from that of the rachis of the ovulate cupules, and it is largely on this evidence that the specific identity of the ovulate cupules and microsporangia is based. The outer cortex of the rachis is composed of fibrous tissue but whether it extends completely round the rachis is uncertain. The inner cortex is parenchymatous and surrounds a central vascular strand of small scalariform tracheids.

In the specimen illustrated (Pl. 1, Fig. 3) each of the two bunches probably contained sixteen sporangia. As most of the sporangia are compressed closely together it has proved rather difficult to determine with any accuracy their size and shape. They appear to be approximately 1·5 mm. long, 0·5 mm. in diameter for about three-quarters of their length, tapering off to an acute apex. They are completely free from one another and are borne terminally in pairs as a result of a dichotomy near the tip of the rachis. The sporangium wall is composed of more than one layer of elongated cells, frequently with dark contents. The petrified sporangia (Pl. 2, Fig. 14) agree in size and shape with those in the compressions, the average dimensions of eight sporangia being: length 1·5 mm., maximum diameter 0·4 mm. Owing to poor preservation of the petrifaction no structure is visible in the sporangium wall.

Although most of the sporangia, both petrified and compressed, have shed their spores there is no indication as to the method of dehiscence. A few spores resembling those present in the lagenostome of the petrified ovules occur inside and in association with the petrified microsporangia but their number is too small to establish beyond doubt that they originated there. In a transfer of one of the compressions, however, it was clear that two of the sporangia had failed to open and on maceration each yielded a mass of several hundred spores (Pl. 2, Fig. 15). In the other specimen all the sporangia appear to have dehisced though a number still contain a few spores and some of these have been isolated (Pl. 2, Fig. 16). In equatorial outline the spores are rounded triangular to circular with a size range of approximately 45 to 58μ, mean 50μ. They have a cingulum which varies from 1·5 to 3μ in width, even in the same specimen; the narrowest regions are usually opposite the rays of the trilete mark. The spore coat is thin; the ornamentation is irregularly punctate. The rays of the trilete mark are straight and extend for about two-thirds of the radius; the sutures are narrow with simple margins. There is no evidence of the presence of any germinal mechanism other than the triradiate mark.

The spores present in the lagenostomes of the petrified ovules are somewhat distorted and even when isolated their original shape and surface features are not clear, though the cingulum is always distinct. The twenty-five spores measured gave a size range of approximately 48 to 60μ, mean 52μ. In spite of the lack of a detailed comparison it seems reasonable to conclude that the spores present in the lagenostomes are identical with those from the microsporangia and that they are in fact the required pollen.

Florin (1937) recognized two basic types of pteridosperm pollen: round, trilete, with or without germinal furrow; and elipsoidal with longitudinal tetrad mark and germinal furrow. Remy (1954) recognized two further types: round, trilete, monosaccate; and rounded-elipsoidal, monosaccate. Clearly the pollen of *Geminitheca scotica* does not fit in with any of these categories and therefore it represents a further type hitherto unknown in the group. Had the pollen grains been encountered as *sporae dispersae* they would probably have been considered to be lycopod miscrospores.

Diagnoses

Geminitheca gen. nov.: Ovulate cupules united in pairs. Cupule divided into two primary lobes, each subdivided into two or three lobes; lobes free almost to base. Cupular vascular strands with abaxial xylem. Two ovules contained in each cupule, borne on short pedicels on cupular lobes. Apex of nucellus differentiated into large lagenostome with salpynx. Integument free to base from nucellus, cupule-like, lobed, lobes becoming free at level of lagenostome and forming long apical processes.

Microsporangia elongated, borne in pairs, but not fused together.

Type species: G. scotica gen. et sp. nov.

G. scotica gen. et sp. nov.: Ovulate cupules borne in bunches containing sixteen cupule-pairs, terminally on naked, dichotomizing rachides. Cupule-pairs 9 to 12 mm. long, united for about a quarter of their length. Maximum diameter of each cupule 2 to 4 mm. Each cupular lobe with adaxial sclerotic and abaxial parenchymatous layers. Two vascular strands passing up abaxial layer. Ovules with three or four prominent longitudinal ridges. Integument six to nine lobed.

Microsporangia about 1·5 mm. long, 0·5 mm. in diameter for three-quarters of their length, tapering to an acute apex. Borne in bunches of about sixteen sporangia, terminally on naked dichotomizing rachides. Microspores rounded triangular to circular in equatorial outline, 45 to 58μ in diameter. Cingulum 1·5 to 3μ broad. Spore coat thin; ornamentation irregularly punctate. Rays of trilete mark straight, extending for two-thirds of radius; sutures narrow with simple margins.

Syntypes. Specimens Pb2674, Pb2680 and Pb2697 in the Hunterian Museum Palaeobotanical Collection, and slides 584–9, 766–8 and 1181–1243 in the Figured Slide Collection, both in the Department of Botany, University of Glasgow.

Horizon. Cementstone Group of the Calciferous Sandstone Series, Lower Carboniferous.

Locality. Tributary on south side of Loch Humphrey Burn, Kilpatrick Hills, Dunbartonshire.

Discussion

It is quite certain that *Geminitheca scotica* is of pteridospermous affinity, but although the ovulate organs and microsporangia agree in general morphology with those of other Palaeozoic pteridosperms it does not appear to be closely related to any previously described genus. In the differentiation of the cupular lobes into an adaxial layer of fibrous tissue and an abaxial parenchymatous layer it shows a development closely parallel to that of *Calathospermum scoticum*, and presumably the opening mechanism suggested for *Calathospermum* (Walton, 1949*a*) would also apply to *Geminitheca*. In dry conditions the parenchymatous outer layer would contract to a greater extent than the fibrous layer and as a result the cupular lobes would become reflexed, thus exposing the ovules for pollination or the fertilized ovules or seeds for dispersal. The cupule does not at any stage appear to have been a tightly closed organ for there are considerable gaps between the lobes even at the base (Text-fig. 4). There is no evidence that the pedicel of the ovule elongated as did that of *Calathospermum* and it is assumed that at maturity the seed merely became detached from the pedicel, since the remains of the latter are present in the empty cupules.

It is quite clear that the cupules are bilaterally symmetrical and that they cannot have been derived from a true whorl of foliar segments. The single collateral vascular strand indicates that the whole bunch of cupules has been derived from a frond or part of a frond that had attained some sort of dorsiventrality, though not necessarily possessing a lamina. The anatomical resemblance between the cupular lobes and the rachis, and the similarity between the branching of the rachis and the divisions which occur in the base of the cupule-pair and in the individual cupules suggest that the cupules arose by the modification of a system of dichotomizing rachides to enclose terminal ovules. This is further borne out by the variation in number of the cupular lobes and the fact that the ovules are actually borne on the lobes. Since usually each lobe possesses two vascular strands it probably represents a segment of rachis in which the vascular strand has forked but the accompanying development of sclerotic tissue and subsequent cleavage have been delayed, and consequently a broad, somewhat flattened structure has developed. The dichotomy is usually completed at the apex of the lobe. No other Palaeozoic pteridospermous ovulate cupules have been described in which the vascular system of the cupular lobes is apparently inverted, and therefore it is unlikely that the cupule of any of these could have arisen in the same way as that of *Geminitheca*. From this evidence, then, it appears that the cupule has been evolved along several independent lines.

Two other fructifications consisting of paired cupules have been described by Arnold (1935, 1939): one, to which no name was given, occurs in the Upper Devonian of Pennsylvania in association with fronds of *Archaeopteris*; the other, *Lagenospermum imparirameum*, occurs in the Mississippian of Pennsylvania and Virginia. Each cupule of *L. imparirameum* contains a single seed, but there is no indication as to the nature of the contents of the Devonian cupules. In both cases the cupules are borne on short stalks and are not fused together, though in some specimens of *L. imparirameum* (Arnold, 1939, Fig. 3) the stalks are so short that the cupules appear as a single unit. Although no structure was described and so it is impossible to make detailed comparisons, neither fructification bears much superficial resemblance to *Geminitheca* and it is very unlikely that they are closely related. Yet certain comparisons may be made between them and *Geminitheca* and *Gnetopsis eliptica* Renault (1885). In *L. imparirameum* the cupules are stalked but there appears to be a tendency to a reduction in the length of the stalks to form a single compound structure; in *Geminitheca* the cupules are completely united at the base and each contains two ovules; in *Gnetopsis eliptica* the cupule is bi-lobed and contains four ovules (Renault, 1885, Pl. 21, Fig. 1). It is not suggested that this is a phylogenetic reduction series but the bi-lobed cupule of *Gnetopsis* is strongly suggestive of an origin from twin cupules similar in form to those of *Geminitheca*. That the cupule of *Gnetopsis* did not originate as a dichotomous structure in the same way as that of *Geminitheca* is obvious from the vascular strands of the cupule, which are orientated with the xylem towards the cupular axis.

The ovules of *Geminitheca scotica* resemble those of *Salpingostoma dasu* and *Calathospermum scoticum* in shape and in the possession of a large lagenostome prolonged at the apex into a tubular salpynx but they differ in possessing a free integument, and in this respect they are more primitive. The similarity between the cupule and the integument suggests that the integument originated by a process analogous to that which resulted in the formation of the cupule: the ultimate ramifications of the rachis system became aggregated around a terminal sporangium and eventually became fused together for about half their length to form the cupule-like integument. These ultimate tips may all have been sterile but the analogy between the hypothetical ovule and the microsporangiate organ rather suggests that initially they may have been fertile and that they became sterile as they assumed a protective function. This recalls the synangial hypothesis of the origin of the integument put forward by Benson (1904). However, there is insufficient evidence finally to decide this point and the question must be suspended until more is forthcoming.

The free integument of *Geminitheca* appears to confirm the view first suggested by Oliver and Scott (1904) that the integument of *Lagenostoma* 'was once a comparatively unspecilised, cupule-like indusium', and also their view, supported by the work of Stopes (1905) on the integument of the Cycadales, that the integument and cupule are fundamentally homologous. It supplies further support to the theory set out by Walton (1953) that 'the integument and cupule in the pteridosperms have each been derived by concrescence of a number of foliar divisions.'

SUMMARY

1. The ovulate and microsporangiate fructifications of a new Lower Carboniferous pteridosperm, *Geminitheca scotica* gen. et sp. nov., are described.

2. The ovulate cupules are united in pairs and borne in bunches terminally on naked, dichotomising rachides. Evidence is put forward to show that the cupules probably arose by the modification of a system of dichotomising rachides to enclose terminal ovules.

3. Each cupule contains two ovules similar in form to those of *Calathospermum scoticum* Walton. They differ in that the integument is cupule-like and free from the nucellus to the base. It is suggested that the integument originated by a process analogous to that which gave rise to the cupules.

4. The microsporangia are also borne terminally in bunches. They are paired but are completely free from one another. The microspores have a distinct cingulum.

5. While agreeing in general morphology with other Palaeozoic pteridosperms *Geminitheca scotica* does not appear to be closely related to any previously described genus.

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EXPLANATION OF PLATES 1 AND 2

(All figures are from untouched photographs)

Key to lettering on the figures: *ec*, emergence on abaxial side of cupular lobe; *h*, multicellular, uniseriate hairs; *hb*, hair base; *la*, lagenostome; *mm*, megaspore membrane; *par*, parenchymatous tissue of cupular lobe; *pr*, apical process of integument; *ps*, remains of prothallus with ?archegonial eggs; *sa*, salpynx; *scl*, sclerotic tissue of cupular lobe; *vb*, vascular strand of cupular lobe.

H.M. Pb, Hunterian Museum Palaeobotanical Collection.

F.S.C., Figured Slide Collection, Dept. of Botany, University of Glasgow.

Plate 1

Fig. 1. Bunch of ovulate cupules compressed laterally. Natural size. H.M. Pb2711.

Fig. 2. Bunch of ovulate cupules compressed more or less vertically. (× 2.) H.M. Pb2680.

Fig. 3. Compression of two bunches of microsporangia. Natural size. H.M. Pb2697.

Fig. 4. Transverse section of the rachis immediately below a cupule pair. (× 30.) F.S.C. 1210.

Fig. 5. Pitted tracheids in rachis: 'pull' of Pb2711. (× 310.) F.S.C. 1219.

Fig. 6. Transverse section of vascular strand in a cupular lobe. (× 100.) F.S.C. 1181.

Fig. 7. Longitudinal section of an emergence on the rachis. (× 56.) F.S.C. 1211.

Fig. 8. Junction of cupule-pair showing hairs. (× 90.) F.S.C. 1200.

Fig. 9. Transverse section of part of a cupular lobe showing an emergence. (× 30.) F.S.C. 1181.

Plate 2

Fig. 10. Stoma on rachis: 'pull' of Pb2711. (× 380.) F.S.C. 1220.

Fig. 11. Longitudinal section of the upper part of an ovule. (× 34.) F.S.C. 1228.

Fig. 12. Megaspore membrane. (× 17.) F.S.C. 1221.

FIG. 13. Oblique section of a lagenostome containing microspores. (× 170.) F.S.C. 1223.

FIG. 14. Longitudinal section of part of a bunch of empty microsporangia. (× 25.) F.S.C. 768.

FIG. 15. Spore-mass isolated from a compression of a bunch of microsporangia. (× 70.) F.S.C. 1242.

FIG. 16. Microspore isolated from specimen Pb2697. (× 500.) F.S.C. 1243.

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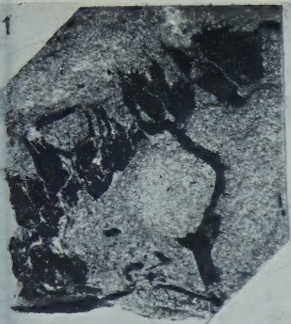

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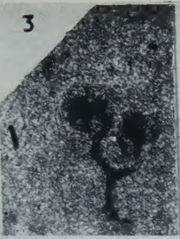

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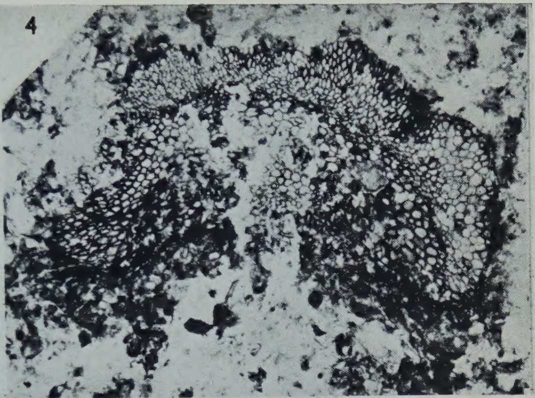

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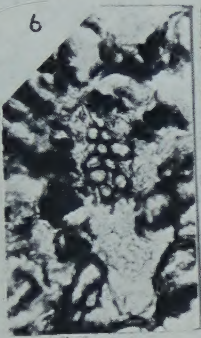

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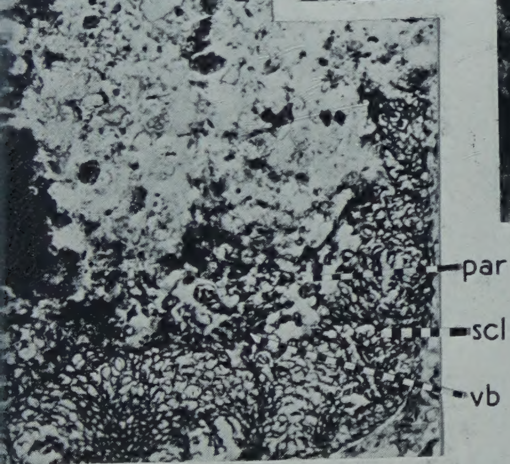

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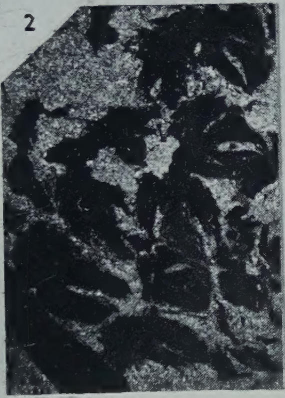

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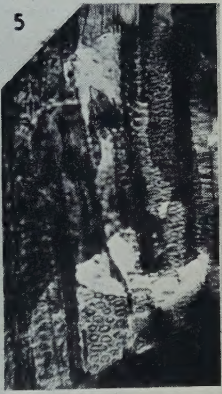

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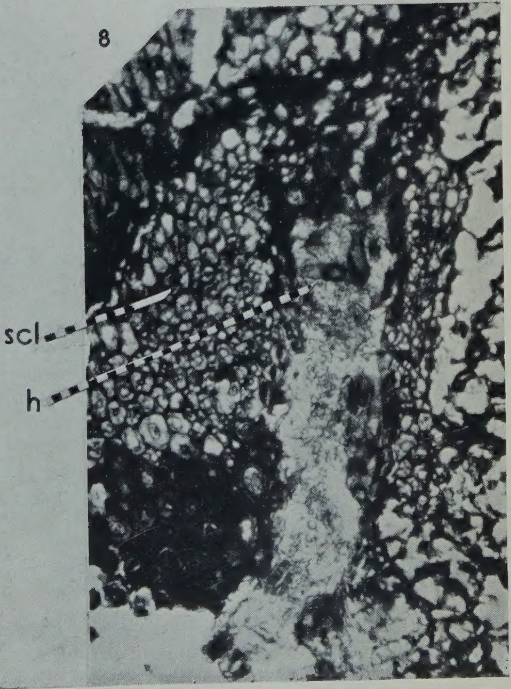

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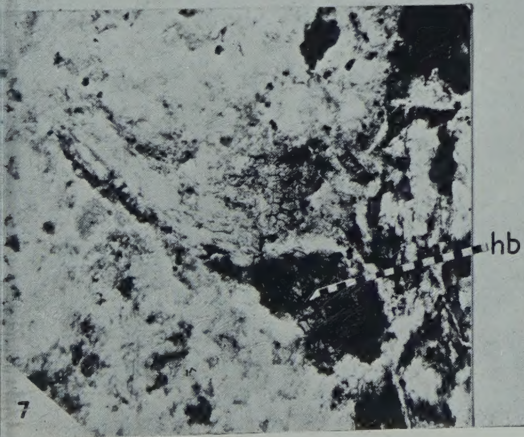

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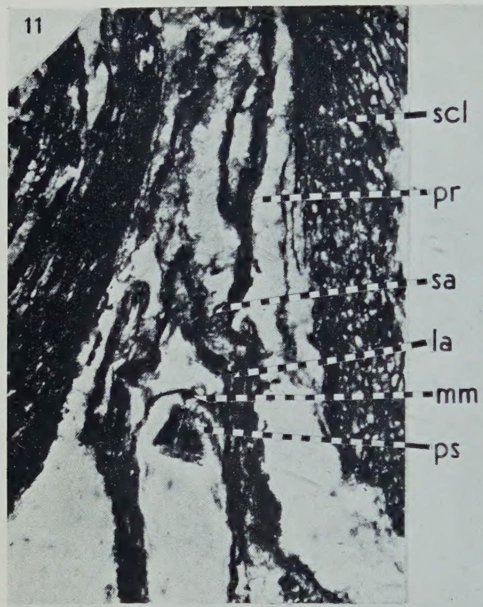
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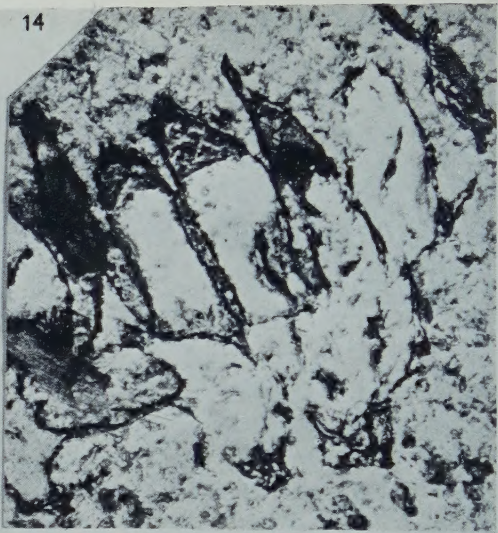
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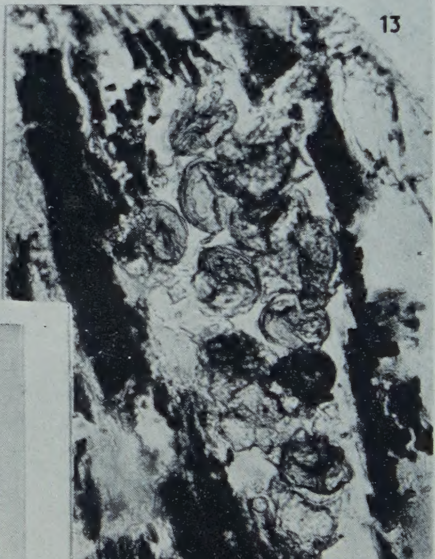
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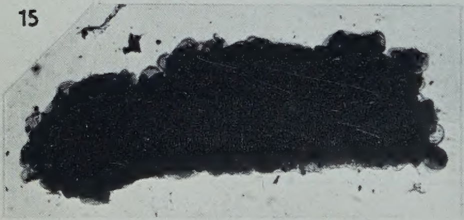
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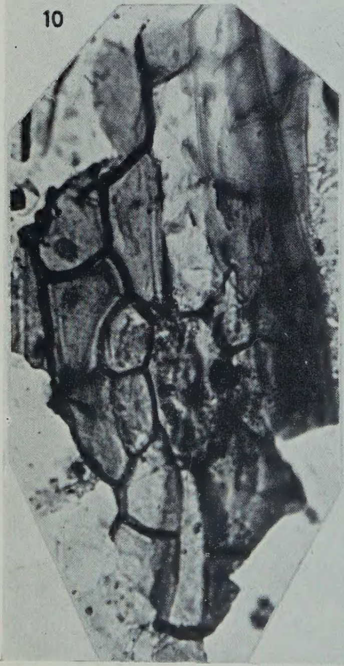
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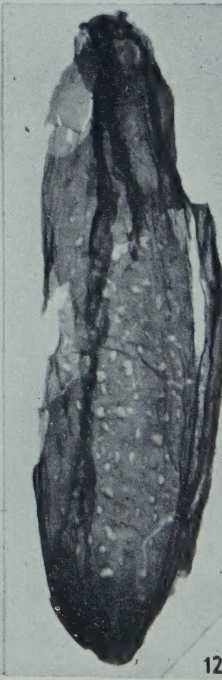
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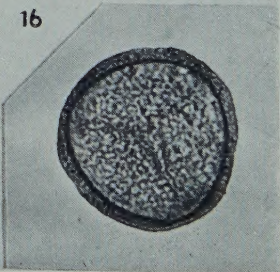
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